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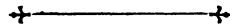
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by
H. MUNRO FOX

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THE TIME FACTOR IN ELECTRICAL EXCITATION

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CONTENTS.

I. INTRODUCTION.

WHEN a constant current is passed through an excitable tissue a process occurs which may culminate in some characteristic response. This process takes time to develop, for if the duration of current flow is made short enough the response will not occur. It is the object of the present article to consider the measurement and significance of the rate of development of this process in various circumstances.

The prime difficulty in this question, as in so many other biological questions, is to know what in fact is to be measured. The excitation process is a composite idea. It involves all those changes occurring in the tissue during the passage of the electric current, which take part in the production of the final response. But these changes should not necessarily have equal weight as characteristic of the excitation process, for some may be supposed to be of prime importance in the process while others have only significance inasmuch as they may affect the prime factors. It thus becomes clear that until we have a theory of the mechanism of excitation far more firmly established than is at present the case, it is quite impossible to seize upon any change of the tissue with confidence that this measures the excitatory process. And again upon the practical side, the change may be so small, so localised and so masked by other irrelevant changes that present technique would be unable to measure the process even though theory had made it clear what in fact ought to be measured.

But though accurate measurement of the development of the excitation process cannot be attempted, at least we know that its total duration in any given case cannot exceed the interval from the initiation of the current to the appearance of the response. Bishop (1928) has shown in nerve that an effective constant current must persist until the instant when the action-potential wave develops, hence in this case

the duration of the excitation process may be considered to be the duration through which the stimulating current must flow. Observation shows (Fig. 1) that this duration is shorter when the current is stronger (Hoorweg, 1892; Weiss, 1901 a, b), hence the excitation process clearly develops faster the stronger the current. It follows that when the rate of the excitation process is to be compared in two different tissues, it is important that the strengths of current should be comparable in the two cases. It is impossible at the present time to assign without arbitrariness any exact meaning to the word "comparable," and it certainly will not necessarily mean that the currents must be the same when measured in the usual way with an electrical instrument, for this measure has no direct relation to the current flowing through the internal structure where excitation occurs. Now for every excitable tissue there
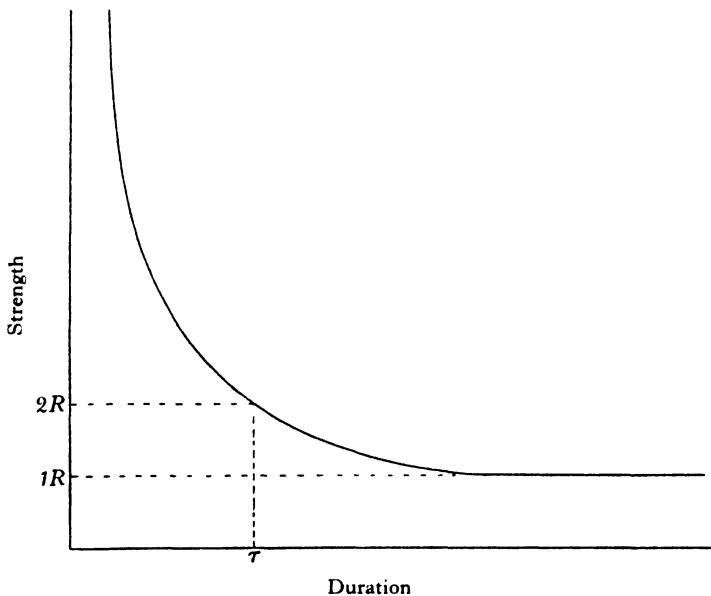
Fig. 1. Diagrammatic strength-duration curve. R = rheobase, τ = excitation time.

exists a finite strength of current below which no excitation will occur however long the current flow. This strength is called the *Rheobase*, and it is usual to assume that currents in various circumstances are "comparable" when they are equal multiples of the rheobase in those circumstances.

With this assumption there now appears a practical and easy way to measure the rate of the excitation process in any given case, namely, to find, first the rheobase, then to take some greater current strength expressed as a multiple of the rheobase (usually double it) and to find the length of time the current must flow in order to elicit excitation. Theoretically it is possible to measure simply the utilisation period of the rheobase itself, and this has been employed in the past, but the measure is a very inaccurate one, since a small error in the exact value of the current strength

produces a large error in the duration to be observed (cf. Fig. 1). The convention now adopted is to find the minimum effective duration where the strength is *twice* the rheobase; this interval of time was called by Lucas "Excitation Time" (1907) and by Lapicque "Chronaxie" (1909). For reasons which will appear later Lucas' nomenclature must now be adopted if we are to avoid confusion.

The widely recognised importance of this measure is partly due to the ease and precision with which a tissue in given conditions may be characterised and partly due to certain claims put forward by Lapicque and his school concerning other properties of the tissue which go hand in hand with the excitation time.

It may in fact be said that to Lapicque and his colleagues more than to anyone else are we indebted for the great advance in precision of excitability measurements which has taken place during the present century. It is not proposed, however, in this article to discuss in any detail the contribution of Lapicque and his school. Many reviews and summaries have in recent years appeared upon this head (Bourguignon, 1927; Coutière, 1928; Lovatt Evans, 1930; Fredericq, 1928; Lapicque, 1926). But since these authors have been concerned rather with the *explanation* of Lapicque's standpoint than with a *critical review*, it may not be superfluous here to give a more critical presentation.

For over twenty years the observations of Lapicque have stood in complete opposition to those of Keith Lucas. All consideration of this opposition and its significance, however, was commonly omitted from reviews and experiments concerned with the chronaxie, but within the last four years this question has been reinvestigated by Lapicque and the writer. The result has been to throw considerable doubt upon the validity of some of Lapicque's former views, and the present article is an attempt to explain the significance of chronaxie measurements in the light of this recent work. At the outset, however, the writer would like to point out that, though he believes the experimental facts quoted to be incontestable, yet the interpretation of them represents his own point of view, and differs widely from the opinion of Lapicque. It is believed that the reader will gain more from the presentation of a single coherent concept, than from a compilation of the opinions of many.

II. LAW OF EXCITATION.

If a tissue is excited by the passage of a constant current of limited duration, then, as the duration is diminished the strength of current has to be increased if the stimulus is to remain just adequate. This relation between current and duration is the well-known *strength-duration curve* (Fig. 1) which may easily be plotted experimentally, but which has so far resisted the very numerous attempts at a complete analytical expression.

Such curves obtained from all kinds of excitable tissue do not differ greatly in general shape and may be made roughly to coincide if they are suitably scaled. The unit of intensity may be conveniently taken as the rheobase in each case, and the unit of time as the excitation time. The fact that all curves when reduced to this scale approximately coincide, strengthens the assumption made above that "comparable"

strengths are equal multiples of the rheobase, and that the excitation time is a characteristic measure of the rate of the excitation process in the given conditions.

Lapicque (1931 *a*) has claimed that the curves coincide not approximately, but exactly. This is not to be expected *a priori* nor is it in fact the case. The curves from medullated nerves differ appreciably from those obtained from slow muscles even when special precautions are taken to avoid inductance errors (Rushton, 1932 *b*). It can be shown, moreover (unpublished), that just such a deviation as that observed might be expected if the exciting current in the one case enters through a gap in the medullary sheath—the node of Ranvier—and in the other through a uniform cylindrical sheath. The complexities of structure arising in cardiac and other tissues make it physically very unlikely that the excitability curves after scaling would all be identical.

If a tissue is excited by the discharge of a condenser, the relation between the initial voltage and the capacity for threshold excitation is somewhat similar to the strength-duration relation. Contrary to a common belief, Lapicque has never claimed the two types of curve to be identical, nor experimentally is this the case.

Lapicque has found (1910) that if the voltage-capacity curve is plotted on the same graph as the strength-duration curve with the same rheobase in the two cases, then, if the unit of time is taken in seconds in the one case and as 3 8 capacity (farads) × resistance (ohms) in the other, the two curves will cut at an intensity of twice the rheobase. It is clear that the duration of this point of intersection is the excitation time of the strength-duration curve, hence this value may be obtained by the condenser technique if the results are scaled as above.

Lapicque has pointed out (1926, Conclusions) that the excitation time not only characterises the rate of the excitation process, but also the rate of conduction of the impulse, the rate of contraction (when the tissue is contractile), the duration of the action-potential wave, the minimum effective rate of slowly increasing current, etc. It would be a mistake to insist upon the exact proportionality (they have different temperature coefficients for one thing (Gasser, 1931)), but certainly excitation time is a good indication of the general rapidity of a tissue.

The simplest rational way of regarding this parallelism between the various phenomena is that suggested by Forbes (1922), that the excitation time represents the rate at which electrical manifestations can develop, dependent, no doubt, upon the electrical constants of the tissue. These same constants operate more or less directly upon the duration of the action-potential wave, the rate of propagation, etc. since all these effects are closely bound up with the development of electrical states in the tissue. From this viewpoint it is easy to see, not only the parallelism, but also the minor divergences which are bound to result from the interplay of factors other than purely electric. It is well known (Cardot and Laugier, 1912) that the excitation time characterises the rate of the excitation process only at the point stimulated. Supposing that this point and its immediate surroundings had the temperature lowered 10° C., then the excitation time would change as though the whole tissue similarly altered in temperature. But the rate of conduction, contraction, and duration of the action-potential wave, measured in the usual way (*i.e.* distant from

the local seat of temperature change) would not show any corresponding alteration. If this local change were not suspected it might be said that the excitation time did not characterise the rate of these other phenomena, but, when one is aware of the situation, the source of error is obvious enough, for the tissue where the excitation time was measured is in a condition different from that where the other phenomena are observed. However, a considerable difficulty arises when it is realised that the very act of stimulating, in order to measure the excitation time, automatically throws the tissue locally into a condition different at least in some respects from that elsewhere. This matter would not be important if the difference were always the same. If the electrode were always 10° C. below the temperature of the rest of the tissue, in the former example, stable comparisons would present no difficulty. The difficulty arises when it is seen that different sizes of stimulating electrodes must involve differently the electrical constants of the tissue and do in fact produce quite different excitation times, though naturally the rate of propagation, of contraction, etc., being independent of these local conditions, suffer no corresponding variation. The question of the size of electrode used to measure excitation time therefore assumes an important theoretical and practical position.

III. SIZE OF ELECTRODES.

When a tissue such as the nerve-free pelvic end of the sartorius muscle is stimulated with electrodes of different sizes it is found that the excitation time is enormously dependent upon the electrode used (Jinnaka and Azuma, 1923; Davis, 1923; Watts, 1924-5; Lapicque, 1932); the larger the electrode (within limits), the longer the excitation time.

In the case of muscle, the excitation time is dependent in this way to the extent of a ratio 1 : 200. In the case of medullated nerve, on the contrary, the measure is not influenced beyond about 1 : 2.

This phenomenon explains the outstanding divergence between the results of Lapicque and Lucas, for the former used small electrodes and obtained brief values for the muscle excitation time, and the latter used large electrodes and obtained long values. But we are left with the difficulty that a measure which is so greatly dependent upon the stimulating technique cannot be characteristic of the tissue unless the technique is standardised. Such a standardisation by itself is merely a matter of technical convenience and reliability and as such is completely arbitrary, but Lapicque believes that the technique which he has used hitherto gives a value which is more correct from a theoretical standpoint.

From the approach adopted in this article there is no physical meaning to the "correctness" of one value rather than another. Lapicque's approach has been from quite a different angle however, and there is no doubt that the centre-piece in his conception of a correct value for the excitation time is his *Law of Isochronism*. According to this law any two tissues which are in such a condition that an impulse can pass from one to the other have the same chronaxie (= "correct" value of excitation time). This law was induced from experiments upon muscle and nerve

using small electrodes. After curarisation, the muscle chronaxie was observed to be considerably prolonged (1906), and as a result of this observation Lapicque formulated his *Theory of Curarisation*, according to which the nerve-muscle conduction was abolished as the result of the fact that the two tissues no longer have the same chronaxies. These conceptions have been urged by Lapicque and his school as fundamental properties of excitable tissues, and a considerable superstructure has been erected upon these foundations.

It is very evident that the excitation times of muscle and nerve by no means coincided when measured by Lucas's technique (1907), and it is only possible to obtain this coincidence (if at all) by using very small electrodes. If, therefore, on other grounds we accept that isochronism is a fundamental property, and if we value the superstructure erected thereupon, it is natural and justifiable to urge the theoretical "correctness" of a technique which enables these results to be maintained.

But the grounds upon which the theory of isochronism and of curarisation rest lie open to grave criticism. The evidence will be treated in some detail later in this article, but to anticipate the conclusions it may be said that it is very doubtful whether any investigator who has satisfactorily distinguished between the excitation of muscle and the excitation of the intramuscular nerve twigs has found nerve-muscle isochronism to hold, and so far from curare paralysing by changing the muscle excitation time, it appears to paralyse without affecting the muscle (or nerve) excitability in any way—in fact it appears to act, as classically supposed, upon some nerve-muscle junction.

In view of these conclusions it is clear that the value of the isochronism theory is not such as to justify any concept of correct value of excitation time, and whether one form of electrode should be used rather than another seems to be a matter simply of reliability and practical convenience. It would be out of place here to enter into any such technical discussion, seeing that experimenters in the field have not begun to agree on the form of electrode which best meets requirements. It may, however, be mentioned in passing that if as the electrodes are made progressively either larger or smaller the excitation time approaches some fixed upper or lower limit, such a limit might conveniently be taken as a practical value. In fact both upper and lower limits have been claimed to occur, but before the technique is standardised, further work is necessary to see whether these limits are relatively independent of the exact position of application of the electrode, nature of contact, degree of moisture on the surface, stability of results with a given setting of electrodes, etc.

Pending the thorough investigation of these conditions it is obviously of prime importance that any investigator making measurements of excitation time should make adequate controls to be sure that the technique employed gives reproducible results and should describe the nature, dimensions, and form of application of his electrodes in sufficient detail for the conditions to be repeated by any readers interested.

IV. THE CHRONAXIE.

Before it was realised that the excitation time of a tissue was dependent upon the form of electrodes used, Lapicque coined the term "Chronaxie," and this name is still widely employed. When it was found that the measure varied in the way that has been discussed, Lapicque insisted that his name, chronaxie, was only to apply to the measure derived from "correct" electrodes (1931 *a*). Other techniques gave pseudo-chronaxies. It appears undesirable to have a terminology so definitely implying that all techniques except one are fallacious, and so Lucas's original name for the measure, "excitation time," is used without any theoretical implication. The chronaxie therefore becomes a particular value of excitation time which fits the criteria put forward by Lapicque.

A question which arises directly from the foregoing is: "What is the mechanism of the dependence of excitation time on size of electrode?" A clear comprehension of this mechanism would obviously go far to remove the foregoing divergence of opinion. The kind of explanation favoured will depend upon whether the subject is approached from the physical or the isochronistic aspect. From the point of view adopted in this article the observed dependence follows intuitively and may be confirmed mathematically as the results of simple physical conceptions. If excitation is supposed to be due to the accumulation of electrically charged particles against a membrane in the tissue under the cathode (as is commonly held) then these will tend to dissipate by diffusion. If the electrode is small there will be much lateral diffusion (like people issuing from a gate into an open space). If the electrode is large there will be little lateral diffusion (a crowd moving from the whole of one side of a square to the other). Consequently with a small electrode the rate of dissipation is much greater and hence the rate of the excitation process must be much greater to overcome it. (For a more logical exposition on these lines see Rushton, 1932 *d*.) A plausible reason why the excitation time of nerve is relatively independent of electrode size is that excitation occurs at the nodes of Ranvier, and these gaps in the insulating medullary sheath are the real micro-electrodes, the phenomenon thus being nearly independent of the gross electrode beyond them.

Lapicque, on the other hand, considers (1932) that there is a single process of excitation going forward at the same rate whatever form of electrode is employed, but that in the case of large electrodes there is a new phenomenon (the α effect) which arises at the electrode, runs a course which is longer the larger the electrode, and that only at the end of this can the excitation proper begin.

The writer has been unable to obtain an exact idea of Lapicque's concept of the α effect and must refer readers to Lapicque's own account. It is clear that the α effect is supposed to be a polarisation which takes longer to mature the larger the electrode. So far his view is in complete agreement with the physical one above. The divergence lies in the supposed effect of the polarisation, for, whereas the physical theory assumes that this polarisation results in excitation, following Nernst and others (Eucken and Muira, 1911; Hill, 1910; Lapicque, 1907; Nernst, 1908;

Umrath, 1924; etc.), Lapicque claims that the polarisation must be complete before the normal and characteristic excitation process can begin.

Without further information, the writer finds it impossible to appreciate this new concept of the excitatory mechanism, and we turn to the consideration of Lapicque's theory of curarisation.

V. LAPICQUE'S THEORY OF CURARISATION.

The most outstanding contribution of Lapicque is his theory of curarisation, which was put forward in the first place to explain the action of drugs such as curare and has since been extended to interpret even the complex phenomena of inhibition and facilitation in the central nervous system (Bremer and Rylant, 1925 a; Cardot, Regnier and Santenoise, 1926; Cardot, Regnier, Santenoise and Varé, 1927 a, b, c, d; Lapicque, 1929). This theory is in the writer's opinion based upon a misinterpretation of experiments, and is actually contrary to certain observed facts; it therefore deserves a somewhat detailed attention in this critical review.

Before the introduction of Lapicque's views, curare was supposed to act by paralysing the myo-neural junction, on the evidence of Claude Bernard. Brücke (1867) had shown that before paralysis the excitation time of the muscle directly excited was the same as that of the nerve, but after paralysis the muscle excitation time became much longer. This he interpreted as due to the fact that initially the nerve twigs were excited, but when conduction from these was abolished the less excitable muscle was itself stimulated and the long excitation time corresponded to the normal value for muscle which initially was obscured by the greater irritability of the nerves.

Lapicque (1906) confirmed the above observations but interpreted them differently. He supposed that initially the muscle had the same excitation time as nerve, but that this was prolonged by the drug so that the value obtained after paralysis was not the normal value for muscle but an abnormal value. He satisfied himself that he could make the final value abnormal by employing enormous doses of curare, finding that in these cases the excitation time was yet more prolonged.

It will be observed that Lapicque gives no actual evidence that initially the muscle excitation time was the same as that of nerve, nor that when *weak* but curarising doses were administered the final muscle excitation time was in any way abnormal.

His strongest argument at this time was that since at the moment of curarisation the muscle excitation time was twice that of nerve and since continued action of strong curare prolonged it, the initial value might well be within the 2 : 1 ratio. This would undoubtedly be the case if drugs which prolonged excitation time in the later course of their action invariably did so initially. Unfortunately this is not the case, for veratrine, for instance, has been shown by Lapicque himself to act on nerve first by shortening the excitation time and later by lengthening it again to the initial value (1912). Clearly a similar action of curare on muscle is consistent with Lapicque's

observations; there are no satisfactory grounds for rejecting the simple interpretation of Brücke.

But not only did Lapicque maintain the supposition that curare changed the muscle excitation time from a value identical with that of nerve to one widely different, but he further claimed that the conduction block was an inevitable consequence of the resulting "heterochronism." This is the climax of his theory, involving as it does the corollary that if an impulse can pass from one cell (or part of a cell) to another, the two must be isochronous.

But this theory of curarisation is even less supported than the supposition of nerve-muscle isochronism which we have been considering, for without the latter it cannot hold, and even granting initial isochronism it by no means follows that the final heterochronism was the cause of curarisation, for it might equally well be an incidental and irrelevant change, possibly due to some other constituent of curare than that which paralyses. This latter possibility must be seriously considered in the light of the observation that the excitation time may change without producing curarisation, and curarisation may occur without any change in the muscle excitation time (see later).

For six years Lapicque's theory rested upon the evidence given above; then a second type of experiment was advanced in confirmation.

It was claimed that strychnine paralysed a nerve-muscle preparation by diminishing the excitation time of nerve without affecting that of muscle (1908). Veratrine on the contrary paralysed by diminishing the excitation time of muscle while producing in nerve the changes mentioned above, ending in a return to normal (1912). It is clear that the application of one of these drugs to a preparation already paralysed by the other should have the effect of diminishing the excitation time of the tissue which is still normal, with the result of restoring an isochronism at a much more rapid value. This, according to the theory, should favour a restoration of conduction.

Lapicque claims that conduction is in fact re-established and constitutes remarkable confirmation of his theory (1912). The writer has made some systematic attempts to confirm the above, as will be described later, but has not succeeded in restoring conduction on any occasion, nor is he aware of any other confirmation of Lapicque's claim.

A third piece of evidence was adduced in 1925 to support the Theory of Curarisation. In this work Lapicque ingeniously makes use of the fact that if a current, instead of starting abruptly, rises gradually to its full value, it will affect a tissue relatively the more strongly the longer its excitation time. Actually he finds that by delaying the rise of current by the use of condensers the effect upon muscle is the same as upon nerve. This he interprets as due to nerve-muscle isochronism, but it may equally be due to the fact that the intramuscular nerve twigs are so much more irritable than the muscle fibres that even this relative favouring of the slower tissue is not sufficient to make it the more prominent. After curarisation the muscle behaves as though it were a slower tissue than nerve, and this Lapicque supposes is due to the effect of the drug on the muscle, but it may equally represent the normal muscle now revealed by the paralysis of the nerves. This third method therefore

goes no further than the first to establish whether initially the excitable substance isochronous with nerve is muscle or in fact nerve itself. And this is all the evidence which has been adduced to support Lapicque's theory.

Before passing to the positive evidence against the theory, it may be worth while to call attention to one or two complications arising from the work of those who support it. The advantage of Lapicque's theory has always been its simplicity. The question of whether conduction between two elements was or was not possible was immediately settled by finding whether the excitation times lay within the 2 : 1 ratio. This simple concept has had to be revised in the light of experiments on strychnine. It is found that if a solution of strychnine is applied to the whole of one nerve-muscle preparation but only to the nerve of a second preparation, the muscle of the first will fail to respond but that of the second will not fail when both are excited through the central ends of the nerve. The classical interpretation that the myo-neural junction is more susceptible than the nerve trunk is inadmissible from Lapicque's standpoint, since he denies the existence of any specialised junctional tissue. According to Lapicque the conduction block in the first preparation is due to the heterochronism between the diminished excitation time of nerve and the normal muscle. But in the second preparation there must be the same diminished excitation time of drugged nerve and the same normal excitation time of the adjacent undrugged portion. Hence there should be the same heterochronism and the same conduction block. This is not the case. The Lapicques took up the investigation of this matter (1913) and put forward the very reasonable suggestion that if the change of excitation time did not occur abruptly but gradually from point to point along the nerve across the boundary of the drugged and undrugged regions, then at no point would there be strictly a heterochronism and hence no conduction block. They found experimentally that there was this gradual transition of excitation time, and so the facts were satisfactorily explained (confirmed by Bremer and Rylant, 1925 b).

But with the acceptance of this explanation we must also accept a very sceptical attitude towards the claims of excitation-time measurement across the junction of nerve and muscle. For the assumption has always been made that the excitation time over all the muscle is uniform and over all the nerve is uniform, and we may certainly question whether during the early course of the application of some drug to nerve the excitation time in the main trunk is the same as that in the small non-medullated termination which is the actual part contiguous to the muscle and hence the part where nerve-muscle isochronism (or its lack) should be measured.

Again, in the work of those who measure the "excitation time" of brain centres and relate them with the measure in peripheral nerves, it will be necessary to take very considerable precautions if we are to be sure that transitional values of the measure along the tracts do not completely invalidate any conclusions concerning facility of conduction and degree of isochronism at the two extremities of the nervous path.

Still more difficult to reconcile with the theory of isochronism is the observation of Mme Lapicque as to the dependence of excitation time of peripheral nerve upon the activity of the higher nervous centres. It is claimed (1923) that when the latter are destroyed or anaesthetised the excitation time of the peripheral nerves is doubled

but that of the muscles supplied remains unchanged. It thus was demonstrated that the Law of Isochronism is valid only for excised preparations and does not apply to animals in possession of their higher centres. Quite recently, however, Lambert, Skinner and Forbes (1933) have seriously called in question these observations of Mme Lapicque.

So far we have considered the theory of Lapicque entirely on the evidence which he and his supporters have adduced, and even so, a critical survey indicates that his case is by no means completely substantiated, and that the difficulties of obtaining a "true chronaxie" on the one hand and of exciting the tissues immediately contiguous to the point where conduction block occurs on the other, transform the technique from a very simple one into an extremely difficult one.

When, however, we turn to the investigations of other workers upon Lapicque's evidence, it certainly appears that curare does not paralyse by altering the muscle excitation time.

In order to settle the matter it is obviously necessary to use a technique which will give the excitation time of muscle without risk of exciting the intramuscular nerve twigs; to apply a weak dose of curare which will paralyse; to find the excitation time of muscle after paralysis and compare this with the value before. Three methods have been employed:

(a) Large fluid electrodes are used (Lucas, 1907; Watts, 1924-5; Rushton, 1933) and the long excitation time of muscle is easily distinguished from the short one of nerve. After paralysis, the muscle excitation time is found to be practically unchanged (exactly unchanged in the experiments of Rushton where the technique was most fully developed).

(b) Small electrodes were used (Boehm, 1910; Rushton, 1933). In Boehm's experiments *nerves* were excited initially; but with his purified curare he found that the muscle excitation time did not alter from the time of paralysis onward even for strong doses. In the experiments of the writer the muscle was stimulated initially as shown by the fact that the excitation time was longer than for nerve, that the small cathode was placed on the nerve-free pelvic end of the sartorius, and the anode placed over the cut end of the nerve where it entered the muscle, and that after the nerves had been paralysed by curare the threshold was not found to be altered for any duration of current. This clearly shows that curare in paralysing doses may have no demonstrable effect at all upon the muscle excitability.

(c) Micro-electrodes were used by Jinnaka and Azuma (1923) and by Grundfest (1932).

The former observed single muscle fibres of the frog's sartorius stimulated directly with a pore electrode at the pelvic end. They observed that curare did increase the excitation time but attributed this to the inorganic salt content. They also found a yet greater change in excitation time as a result of bathing in a Ringer's fluid containing 0·046 per cent. KCl. The results are not conclusive however, as no evidence is given as to whether curarisation actually occurred or not in the two cases.

The experiments of Grundfest, however, were well controlled. He worked with

micro-injection capillary tubes applied to single fibres in the frog's retrolingual membrane. Even when pores of diameter 20–60μ were used, the excitation time of muscle was still found to be three times as long as that of nerve, so that exceedingly small electrodes must evidently be used to ensure isochronism. The excitation time of a muscle fibre was found unchanged before and after the action of a dose of curare which was shown to have abolished nerve conduction to that fibre.

It is clear that all this work goes to show that the doubts expressed earlier as to the validity of Lapicque's interpretations of his experiments were well founded, and this later work, by avoiding the ambiguity of the earlier experiments has proved definitely that, whatever may be the mechanism of curare poisoning, it is not essentially through an alteration in the muscle excitation time, no matter what kind of electrodes are used to measure it.

With regard to the support for Lapicque's theory derived from the alleged strychnine-veratrine antagonism, little further need be said. It is obvious that this antagonism could not prove the theory, but only confirm it when already established on other grounds. But as has just been seen, those other grounds are wanting, and hence the drug antagonism, even if it invariably occurred, would have to be explained in some other way. However, in the experience of the writer the antagonism, so far from being invariable, has in no single instance been observed. The attempts to demonstrate the restoration of conduction after paralysis by one drug through the addition of the other (Rushton, 1933) need not be mentioned here, as in any case this drug antagonism can lend little weight by itself to Lapicque's theory.

The last argument of Lapicque is that which relates to the passage of slowly increasing currents. It was pointed out earlier that though this method favoured the excitation of muscle it could not be taken for granted that even so, nerve was not excited owing to its inherent greater excitability. This question must therefore be treated on the same lines as the question of excitation by abrupt currents. The sartorius was excited at the pelvic end with the same arrangement of electrodes as in case (b) above, but with an electric circuit which retarded the establishment of a current by means of a condenser (Rushton, 1933). The results were in complete agreement with the former ones, and contradictory to the *interpretation* of Lapicque. For the time relations of uncurarised muscle were very different from those of nerve, and after curare the muscle excitability was in no way affected.

But this conclusion, which fits so completely all the other work which has clearly differentiated between the initial excitation of muscle and of nerve, is in no way contradictory to Lapicque's *observations*. For if he were initially stimulating nerves, and later when these were paralysed, muscles, this would account for all his observations.

In conclusion we may state, first that Lapicque's evidence did not inevitably lead to his conclusion, second that further work has favoured the alternative interpretation. Thus heterochronism is not of importance in the production of a conduction block, and isochronism of muscle and nerve (if it ever occurs) is merely incidental to the type of electrode used.

A paper by Lapicque has just appeared (1934) in which he forcefully attacks the above criticism of his theories. It would be out of place here to enter into a detailed discussion of this new publication, but three points may briefly be mentioned, since they bear strongly upon the foregoing section.

(*a*) He publishes for the first time in detail his 1906 experiments upon which his Theory of Curarisation was founded, and from these figures it is clear that his technique was excellent and that he had good grounds for concluding that he was measuring the muscle excitability itself and that the chronaxie lengthened on curarisation.

(*b*) From 1912 until quite recently Lapicque and his colleagues have believed that veratrine always curarised by *reducing* the muscular chronaxie, but it has now been shown that the chronaxie may become either shorter or longer. In the latter event conduction will not be restored by strychnine, and no doubt this was the condition in all the experiments of the writer. This satisfactorily clears up one divergence of observation.

(*c*) An attempt to clear up a second discrepancy, unfortunately, cannot so easily be accepted. It is ingeniously suggested that if in the writer's experiments upon curarisation the muscle chronaxie was already very near the 2 : 1 limit before the drug was added, it would only require a very little extra slowing to achieve this limit. Against this explanation are the facts, first that there was never any evidence of spontaneously impaired conduction either in these or any other comparable experiments, second that no matter whether curare was added shortly after excision or several hours later a stimulus to the nerve always gave at first a vigorous twitch, which declined to zero in about 7 min. Third, there was absolutely no lengthening of muscle chronaxie accompanying this change from vigorous twitch to complete block.

On the evidence of (*a*) above it is clear that curarisation may be accompanied by a progressive lengthening of muscular chronaxie. On the evidence of (*c*) it equally appears that this is not necessarily the case. It is to be hoped that work in the near future will reveal the conditions determining these two alternative results (as Lapicque has done for the strychnine-veratrine antagonism). In the meantime it would seem difficult to exclude the observations that in certain conditions curarisation may occur without chronaxie change, and hence the conclusion that conduction block cannot necessarily be due to this change.

VI. THE SIGNIFICANCE OF THE EXCITATION TIME.

The doubt which has been thrown upon Lapicque's theory of curarisation has naturally robbed the excitation time of much of its importance. When it is also borne in mind that this measure may be in the highest degree dependent upon the size of electrode used and that in any case it will only characterise the rate of the excitation process in the conventional sense set forth at the outset of this article, then the question naturally arises as to what is the value, theoretical or practical, of the excitation time. From the theoretical aspect it is obvious that the strength-duration curve is intimately bound up with the time curve of the excitation process, and with the questions as to why in one tissue this process develops faster than in another, and why the measure may be so largely dependent upon electrode size. All these questions are important and fundamental, but it is rather with the practical aspect that we are here concerned. In this domain the outstanding advantage of excitation time is to distinguish which of various possible tissues is being excited in given circumstances.

If, for instance, we stimulate a vertebrate muscle directly, and wish to know whether the muscle fibres or the intramuscular nerve twigs are being excited by

threshold currents, the excitation time may give the answer at once. For if the value is greater than that admissible for nerve (*e.g.* 0·001 sec. as an upper limit for the frog at room temperature), then muscle must be the tissue in question. If large electrodes are used the converse can also be stated with a high degree of probability, *i.e.* excitation times within the limits admissible for nerve indicate that nerves are in fact being excited. (Evidence for this identification is given in Rushton, 1932 *c*.)

But if such an analysis is to have any significance whatever it is essential to obtain the whole strength-duration curve and not simply the rheobase, and then the minimal effective duration at double this intensity, *i.e.* the excitation time.
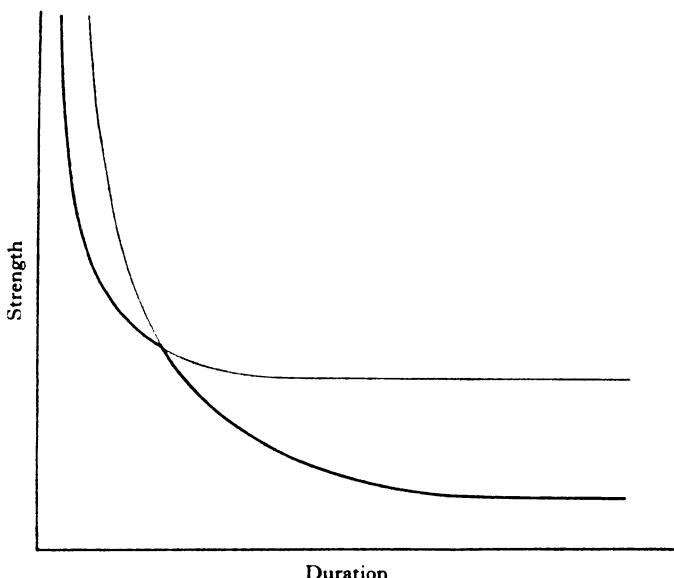
Fig. 2. Diagrammatic strength-duration curves of two different excitabilities. The curves intersect but only the heavily-inked portions are experimentally obtained.

It often happens that the strength-duration curve is not smooth but shows a kink (Fig. 2). In this case the explanation (Rushton, 1930; Grundfest, 1932) is that there are two curves due to two different excitabilities. These curves cross, but since the essence of *threshold* measurement is to record the lowest intensity that gives any response, the observed curve is as shown by the heavy lines, the light lines not being demonstrated without some elaboration of the technique. It is clear that in the above case the rheobase will lie on the slower curve. Twice the rheobase may cut the complex curve below the kink or above it, depending where the kink chances to lie. If below it, the excitation time will be that of the slower curve without error; the faster curve does not enter; but failure to obtain the whole curve results in failure to observe that there is a kink and hence failure to realise that not one but two excitabilities are present. If, however, the kink occurs below the level corresponding to twice the rheobase, the minimum effective duration is neither the excitation time

of the slower nor of the faster excitability but is a complicated and non-significant measure compounded of the excitation times of both, together with the relative intensities of the two rheobases. This measure is useless enough when we recognise what it is, but it is positively misleading when it is called the excitation time of the single tissue supposed to be involved. Slight relative changes in rheobase (such as may easily result from little movements of the electrodes favouring one tissue more than the other) are interpreted as changes in excitation time, and drugs which affect the excitation time of neither but which affect the rheobases to different extents, again are said to alter excitation time.

All these errors may easily be avoided. If from other information we know that only one excitability is in question, then no kinks can be involved and the simple measurement of excitation time by two determinations is legitimate. Again, if the whole strength duration curve is smooth and if there is reason to believe that no subsequent changes will involve kinking of this curve, the simple determination may also be employed. But in the presence of a curve with a kink, or with the possibility of a kink, the whole curve must be determined, and the two excitabilities revealed must be considered separately.

The whole curve may usually be obtained accurately enough as follows. First find the rheobase, and its minimum effective duration (= utilisation period). Now, find the thresholds corresponding to the following fractions of the utilisation period, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{10}$, $\frac{1}{20}$, ($\frac{1}{30}$). It is useful to plot the results as they are found, and then if a kink is in question, one more observation corresponding to the duration where the kink is suspected usually clinches the matter. Logarithmic scales are better than uniform ones for this analysis (Rushton, 1931).

The importance of obtaining the whole strength-duration curve has been specially emphasised because this technique has been omitted in most of the work of Lapicque, Bourguignon and their colleagues. From the isochronistic standpoint it is unnecessary to consider kinks, for if two excitabilities are present they will either act upon different effector mechanisms, *e.g.* the snap muscle and the grip muscle in the claw of *Homarus* (Lucas, 1906) or of *Astacus* (Lucas, 1917), in which case the distinction can probably be made by observing the type of response without the analysis of the strength-duration curve. Or the two excitabilities will act upon the same effector in which case (according to the theory) they must have the same excitation time and consequently cannot give rise to a kink. Without returning to any criticism of this theory, it may simply be remarked that if it is found that there is never a kink, then that is a condition (as stated above) when the simple excitation time technique may be employed, but since many investigators have found that kinks are in fact observed, it will be worth while at least making the control in all doubtful cases, before drawing conclusions which a kink may render so very far from the truth.

Thus the measurement of excitation time may be used to differentiate between two contractile mechanisms (Lucas, 1906, 1917), between two excitable systems acting upon the same contractile mechanism (Lucas, 1907; Watts, 1924-5; Grundfest, 1932), to study the nature of loss and subsequent regain of function in human nerve palsies (Adrian, 1917), to investigate the action of drugs and of other conditions

(Laugier (1929) gives eighty-six references in this category). A vast amount of observations has been made both in physiology and in medicine upon the variation of excitation time in all sorts of circumstances, but in physiology a large proportion and in medicine nearly all the conclusions are rendered uncertain by the single omission of a control to find whether one, or more than one, excitability was involved in the observations.

If in future such a control is made we may well expect that changes in excitation time will turn out to be less capricious and more significant than has hitherto been claimed when every relative variation in the rheobase of muscle and nerve was hailed as a chronaxie change. But if the result of a couple of observations taken without analysis continues to be called "chronaxie," then to determine this chronaxie will be to perform an empty ritual.

VII. SUMMARY.

The rate of development of the excitatory process in tissues may conveniently be characterised by a measure, the "Excitation Time," which was formerly known as "Chronaxie." This constant also measures roughly the rate of other processes in the tissue, *e.g.* action-potential wave, conduction velocity, rate of contraction, etc. probably because all these processes are limited by the development of electrical states in the tissue.

The excitation time of muscle (but not medullated nerve) is very largely dependent on the size of the electrodes used. This is easily explained in terms of a physical theory of excitation. If nerves are stimulated by the penetration of current through the nodes of Ranvier, these will constitute unvarying pore electrodes and account for the relative independence of nerve upon electrode size.

According to Lapicque, paralysis by curare and other similar conditions is due to a great increase in chronaxie of the muscle, which initially was the same as that of the nerve. This standpoint, which is fundamental in Lapicque's school, is criticised in detail. In the first place, the evidence upon which the theory rests is inadequate. In the second, further work by Lapicque's school has rendered the theory so complicated that it is now of doubtful practical value. Lastly, the experiments of other workers appear to make the theory untenable.

In the light of the rejection of Lapicque's views and of the dependence of excitation time upon the nature of the electrodes, it is necessary to review the significance of the measure, and to modify the technique of its determination. Some practical aspects are discussed, and it is pointed out that at present the chief application to biology is in the analysis of multiple excitabilities (*e.g.* muscle with nerve twigs, tonic and phasic muscles, etc.). But it must be emphasised that in this analysis it is essential to determine the whole strength-duration curve if the results are to be significant.

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ÜBER DAS ZEITGEDÄCHTNIS BEI TIEREN

VON

I. VON STEIN-BELING.

INHALT.

I. EINLEITUNG.

VIELE Organismen folgen in ihren Lebensgewohnheiten dem periodischen Wechsel der Jahreszeiten, dem Rhythmus von Tag und Nacht, Ebbe und Flut. Diese Gesetzmässigkeiten sind in manchen Fällen zu Induktoren "biologischer Rhythmen" geworden. Bei der Ausschaltung der Induktoren treten sie vielfach noch eine Zeitlang selbständig in Erscheinung, als "Zeitgedächtnis." Als ein einfaches Beispiel hierfür ist der regelmässige Wechsel von Schlaf und Wachen zu nennen, der bei einem überwiegenden Teil aller Lebewesen dem Wechsel von Tag und Nacht angepasst ist. Wenn ein solcher Organismus unter Ausschluss des periodischen Lichtwechsels gebracht wird, so "erinnert" er sich dessen meistens noch einige Zeit, d. h. er behält den 12 : 12-stündigen Wechsel von Ruhe und Aktivität zur gleichen Zeit bei, in der sich der Wechsel von Tag und Nacht vollzieht.

Es gibt auch Lebensrhythmen, deren auslösende Faktoren uns noch völlig unbekannt sind. Hierzu gehören die tagesperiodischen Schwankungen der Körpertemperatur, des Blutdruckes, des Gaswechsels und der Nierensekretion. Völker (1927), der diese Abläufe am Menschen untersucht hat, schreibt ihren regelmässigen

periodischen Ablauf der Einwirkung eines uns noch unbekannten tagesperiodischen Faktors zu.

Es sollen im Folgenden die Fälle geschildert werden, in denen auf Grund von Beobachtungen und Versuchen ein Zeitgedächtnis festgestellt werden konnte. Dabei soll mit der primitivsten Form dieses Zeitgedächtnisses begonnen werden, den Rhythmen, die in deutlicher Beziehung zu Tagesperiodizitäten stehen und nach deren Ausschaltung langsam erlöschen. Sie sind nicht variabel, wie wir dies z. B. bei dem Zeitgedächtnis der sozialen Insekten beobachten können. Über das Wesen der biologischen Rhythmen ist man sich noch nicht klar. Es wird einerseits angenommen, dass es sich um eine Vererbungserscheinung handelt, andererseits soll auch eine individuell erworbene "Erinnerung" an den periodisch wiederkehrenden Reiz möglich sein.

II. KOSMISCHE RHYTHMEN IN IHRER EINWIRKUNG AUF LEBEWESEN.

(1) *Gezeitenwechsel.*

Der Eintritt des Gezeitenwechsels zwingt viele Meeresorganismen zu Schutzreaktionen. So schliesst sich die Seeanemone *Actinia equina* in der Gezeitenzone nach Bohn (1906) bei Eintritt der Ebbe, um nicht auszutrocknen. Bei Eintritt der Flut werden die Tentakel wieder geöffnet. Bohn fand, dass dieser Rhythmus sich nach dem Verbringen der Tiere in ein Aquarium noch einige Zeit erhält. Obwohl im Aquarium weder Ebbe noch Flut herrscht, waren die Aktinien noch zwei bis drei Tage lang genau zur Ebbezeit kontrahiert, zur Flutzeit entfaltet. Dann verwischte sich der Rhythmus langsam, doch ein Kunstgriff genügte, um das Zeitgedächtnis wieder aufleben zu lassen. Ein leichter Stoss an das Aquarium zur Ebbezeit veranlasste Schliessen, zur Flutzeit Öffnen der Tentakel. Aktinien, die ihren natürlichen Standpunkt an Orten haben, wo sie zu jeder Zeit gleichmässig von Wasser bedeckt sind, zeigen nach Pieron (1906) im Aquarium keine rhythmischen Bewegungen. Pieron und Bohn (1910) erklären sich diese Tatsachen aus der verschiedenen Herkunft ihrer Versuchstiere. Jedoch zeigt nach Crozier (1921) die Aktinie *Actinia bermudensis* kein "Gezeitengedächtnis" im Aquarium, obwohl sie in der Gezeitenzone lebt. Diese Fälle bedürfen also noch der weiteren Untersuchung.

Bei einer Küstendiatomee wird von Fauvel und Bohn (1907) ebenfalls von einem Zeitengedächtnis berichtet. *Pleurosigma aestuarii* verkriecht sich unter natürlichen Bedingungen etwa zwei Stunden vor der Flut in den Sand. Zwei Stunden vor der nächsten Ebbe erscheint sie wieder an der Sandoberfläche—wohl getrieben von Sauerstoff- und Lichtmangel. Im Aquarium liess sich dieser Rhythmus genau gleichzeitig mit den Gezeiten noch eine Woche lang beobachten. Von Würmern (Convoluten) werden von Bohn (1910) ähnliche Erscheinungen beschrieben, die aber umstritten sind (Keeble und Gamble (1903) und Pieron (1910)).

Auch Schnecken sollen ein "Gezeitengedächtnis" haben. Durch die Einwirkung von Ebbe und Flut werden die Tiere in einem Zeitraum von etwa 12 Stunden sechs Stunden lang von der Dunkelheit und sechs Stunden lang vom Licht angezogen. Auch im Aquarium gehaltene Tiere zeigen noch dasselbe Verhalten. Dagegen sollen

nach Bohn (1910) Littorine, die auf höher gelegenen Felsen wohnen und während der Nippflut trocken liegen, nicht so sehr eine tägliche, als eine halbmonatliche Periodizität zeigen. Diese Schnecken werden von der gewöhnlichen Ebbe und Flut nicht berührt: nur während der Nippflut liegen sie trocken und ziehen sich dann in ihr Gehäuse zurück. Bei der Springflut kriechen sie wieder umher. Nach Bohn verhalten sich diese Schnecken im Aquarium ebenso, doch verliert sich dieses Gedächtnisphänomen, für das Bohn keine Erklärung weiss, nach kurzer Zeit vollkommen.

Von Krebsen berichtet Drzewina (1910), dass der Einsiedlerkrebs *Clibanarius misanthropus* ein Gedächtnis für den 14-tägigen Gezeitenwechsel besitzt. Der Krebs ist an der Küste des Atlantischen Ozeans bald positiv, bald negativ phototaktisch, und zwar genau gleichzeitig mit dem Gezeitenwechsel. Zur Zeit der Nippflut sind die Tiere zwischen 12 und 6 Uhr an dem Beobachtungsplatz nur von einer dünnen Wasserschicht bedeckt, und verkriechen sich deshalb zum Schutz gegen die Einwirkung der Sonne unter die Felsen. Zur Zeit der Springflut dagegen sind sie am gleichen Beobachtungsplatz zu den gleichen Stunden durch eine genügend hohe Wasserschicht geschützt: daher verkriechen sie sich nicht, sondern wandern häufig noch in die Höhe. In ein zur Hälfte verdunkeltes Aquarium versetzt, zeigen die Krebse einen 14-tägigen Rhythmus, eine deutliche Erinnerung an die Gezeiten: zur Zeit der Nippflut wandern sie in den verdunkelten Teil des Behälters, zur Zeit der Springflut in den hellen Raum. Von besonderem Interesse ist die Feststellung, dass auch bei *Clibanarius misanthropus* Unterschiede in der Reaktion, je nach dem Standort der Tiere zu beobachten sind, wie sie Bohn von den Aktinien berichtet hat: wie Drzewina mitteilt, zeigen die Krebse an der Küste des "gezeitenlosen" Mittelmeeres keinerlei negativ phototropischen Bewegungen. Diese Beobachtung spricht für ein auf assoziativem Wege erworbenes Zeitgedächtnis.

(2) *Mondphasen.*

Hier soll eine Erscheinung besprochen werden, die viele Forscher beschäftigt hat, ohne bis heute eine eindeutige Lösung gefunden zu haben. Die unter der Überschrift "Gezeitenwechsel" berichteten Rhythmen lehnten sich deutlich an die Gezeiten als solche, soweit sie mit den Bewegungen des Wassers zusammenhängen, an. Hier konnte keine direkte Beziehung zu dem die Gezeiten auslösenden Gestirn, dem Monde und seinen Phasen gefunden werden. Ein ausgesprochenes Mondphasen-Zeitgedächtnis dagegen zeigen Vertreter der Polychäten. Das Auftauchen des "Palolo" bildet mit seiner Pünktlichkeit das bekannteste Beispiel. Der "Palolo" ist die reife Körperhälfte der Würmer, die während der durch Monate dauernden Geschlechtsreife der Polychäten sich vom Körper ablöst und an die Meeresoberfläche emporsteigt. Dies geschieht während der ganzen Zeit der Geschlechtsreife bei ganz bestimmten Mondphasen, und zwar hat jede Polychätenart ihre bestimmte Mondphase. Selbst bei Neumond schwärmen bestimmte Polychäten, gewisse Formen sogar bei Vollmond und Neumond zusammen. Für dieses Phänomen fehlt bisher jede ausreichende Erklärung. Die Lichtintensität der verschiedenen Mond-

phasen ist nicht von entscheidendem Einfluss auf das Schwärmen, denn Polychäten, die nicht auf Neumond eingestellt sind, schwärmen auch bei vollständig bedecktem Himmel (Hoffmann 1926). Auch die Schwerkraft, Sonnenwärme, Salzgehalt des Wassers und luftelektrische Spannungen wurden durch Untersuchungen als die auslösenden Faktoren ausgeschlossen. (Fage und Legendre, 1923; Herpin, 1924; Hempelmann, 1911; Friedländer, 1899[1].) In einem Korallenriff ins Aquarium verbrachte Polychäten (*Eunice*) schwärmten unter diesen konstanten Bedingungen genau zu derselben Zeit, wo im Meere das Aufsteigen des "Palolo" ihrer Art erfolgte. Brunelli und Schoener (1904) erklären sich dieses Zeitgedächtnis damit, dass sie einen sexuellen Rhythmus annehmen, der ursprünglich parallel mit äusseren Einflüssen, als periodisch wiederkehrende "Nervenkrise" entstanden ist.

Über die Beziehung zwischen Mondphasen und Reifezustand der Gonaden liegen weiterhin von Fox (1923) Untersuchungen vor. Fox fand, übereinstimmend mit den Aussagen der dortigen Bevölkerung in Suez, dass der Seeigel *Centrechinus setosus* im Roten Meer seine Reife kurz vor Eintritt der Vollmondphase erreicht. Während der mehrere Monate dauernden Schwärmzeit werden die Geschlechtsprodukte stets zur Zeit des Vollmondes entleert. Bei abnehmendem und wieder zunehmendem Mond bilden sich dann langsam wieder reife Geschlechtsprodukte, die nun wieder bei Vollmond ausgestossen werden. Dieser Vorgang wiederholt sich bei ein und demselben Individuum während der ganzen Schwärmzeit.

Bei dem im "gezeitenlosen" Mittelmeer lebenden Seeigel *Strongylocentrotus lividus* konnte Fox keine Beziehung zu einer bestimmten Mondphase finden, obwohl die dortige Bevölkerung glaubt, dass eine solche besteht. Dagegen beobachtete Fox, dass die Ausstossung der Geschlechtsprodukte bei beiden Geschlechtern stets zu gleicher Zeit, aber in unregelmässigen Abständen erfolgt. Was für Gründe hierfür massgebend sind, konnte noch nicht ermittelt werden. Die Ursachen des periodischen bezw. gleichzeitigen Entleerens der Geschlechtsprodukte bei den Meeresorganismen sind allgemein noch nicht erforscht. Fox untersuchte mehrere in Frage kommende Faktoren (Nahrungsaufnahme, Wasserstand, Wassertemperatur) ohne positives Ergebnis. Auch der Salzgehalt des Wassers, und der Zustand der Meeresoberfläche sind nach seiner Meinung nicht die Ursachen. Dass der Mond bei der Geschlechtsreife von *Centrechinus* die wesentliche Rolle spielt, ist nach Fox' Untersuchungen ohne Zweifel. Doch sind die Zusammenhänge, ebenso wie bei dem "Palolo" noch ganz unbekannt. Die von anderer Seite ausgesprochene Vermutung, dass das teilweise polarisierte Mondlicht die Geschlechtsprodukte beeinflusst, trifft nach Fox' (1932) Ansicht ebenfalls nicht zu. Ebensowenig hält er es für möglich, dass die Lichtmenge des Mondes *zusammen* mit dem aufgenommenen Tageslicht von Einfluss ist.

Einige andere von Fox untersuchte Meerestiere, Vertreter der Muscheln (*Mytilus*) und Krabben (*Neptunus*), zeigen weder im Mittelmeer noch im Roten Meer eine Mondperiodizität, obwohl die Küstenbewohner glauben, dass diese essbaren Tiere zur Zeit des Vollmondes am fleischigsten sind. Bei *Pecten opercularis*

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[1] Auch Kafka (1914) bringt eine ausführliche Schilderung der einschlägigen Arbeiten.

konnte Amirthalingam (1928) Beobachtungen über einen Zusammenhang zwischen Vollmond und Reife der Geschlechtsprodukte machen.

(3) *Tag- und Nachtwechsel.*

Dem 12-stündigen Tag- und Nachtwechsel sind zahlreiche biologische Rhythmen zeitlich angepasst. Ihre Umkehr ist in den meisten Fällen nicht möglich. So ist es Tatsache, dass bei Personen, die durch ihren Beruf gezwungen sind, nachts in Tätigkeit zu sein, und tagsüber zu ruhen, die organischen Lebensvorgänge dadurch nicht verändert werden. Puls, Blutdruck, Gaswechsel und Nierensekretion behalten auch bei dieser umgetauschten Lebensweise ihren tagesperiodischen Verlauf bei, wobei die Maxima und Minima der Verlaufskurven in die gleichen Stunden fallen, wie bei Personen, die in ihrer Lebensweise dem gewohnten Wechsel von Tag und Nacht unterworfen sind (Völker, 1927). So sollen z. B. Krankenschwestern trotz ausgedehnter Tagesruhe bei den Nachtwachen ihre nächtliche Müdigkeit und Schlafbedürfnis in der Regel nicht verlieren. Will man bei diesen Erscheinungen nicht einen uns noch unbekannten tagesperiodischen Faktor als den Urheber ansehen, wie es Völker tut, so muss man dieses Zeitgedächtnis des Organismus in einer inneren "Uhr" suchen, die die organischen Abläufe reguliert. Neuere Untersuchungen lassen die Vermutung zu, dass es sich hierbei eventuell um Stoffwechselvorgänge handelt, wie sie bei Bienen und anderen sozialen Insekten bereits als die "Uhr" des Organismus erkannt worden sind.

Über die Verteilung der Ruhe und Aktivität bei Tieren im Rahmen des 24-Stundentages verdanken wir Scymanski (1914, 1916, 1920, 1922) wertvolle Untersuchungen. Er konnte feststellen, dass jede Tierart eine bestimmte, für sie charakteristische Anzahl und Verteilung von Ruhe- und Aktivitätsperioden im Laufe von 24 Stunden besitzt. Dabei unterscheiden sich deutlich zwei Gruppen voneinander: die der monophasischen und die der polyphasischen Tiere. Zu der ersten Gruppe gehören vorwiegend "Augentiere," auch der erwachsene Mensch. Charakteristisch für die monophasischen Tiere sind zwei Hauptperioden: eine längere Ruheperiode während der Nacht und eine grosse Aktivitätsperiode während des Tages. Die polyphasischen Tiere folgen dagegen im Wechsel von Ruhe und Aktivität nicht dem Wechsel von Tag und Nacht. Zu ihnen gehören interessanter Weise vorwiegend die osmatischen und taktilen Tiere, auch der Mensch im Säuglingsalter. Die Ruhe- und Aktivitätsperioden der polyphasischen Tiere lassen keine Beziehung zum Wechsel von Tag und Nacht erkennen.

Scymanski stellte einige Versuche an, die im Rahmen des Themas von besonderem Interesse sind. Er versuchte ein ausgesprochen monophasisches Tier, den Kanarienvogel (*Serinus canaria*), in seinem gewohnten Tagesrhythmus zu stören, indem er ihn 73 Tage hindurch in dauernder Dunkelheit hielt. Es zeigte sich dabei, dass der Vogel dennoch eine "Erinnerung" an die Zeiten hatte, in welche seine Ruhe- und Aktivitätsperioden unter normalen Umständen fielen. Trotz dauernder Dunkelheit erwachte der Vogel morgens zur gewohnten Zeit, und erst im Laufe von 73 Tagen hatte sich der Beginn der morgendlichen Hauptaktivitätsperiode von

5 Uhr 30 Minuten auf 11 Uhr 45 Minuten a.m. verschoben. Die Länge der Aktivitätsperiode verkürzte sich in der langen Dunkelhaft nur um eine Stunde. Hier haben wir ein Zeitgedächtnis, von dem vorderhand nicht beurteilt werden kann, ob es sich dabei um Gedächtnisleistung, ererbten Rhythmus oder um den Einfluss eines im Dunkel noch wirksamen Aussenfaktors handelt. Als das Versuchstier von Scymanski wieder dem Tag- und Nachtwechsel ausgesetzt wurde, trat der normale monophasische Rhythmus wieder klar zutage.

Nach Darwin (1882) sollen Regenwürmer (*Lumbricus terrestris*) ein genaues Zeitgedächtnis für den Wechsel von Tag und Nacht besitzen. So konnte von ihm beobachtet werden, dass die Tiere ihre Gewohnheit, tagsüber in ihren Röhren zu verbleiben und sie erst nachts zu verlassen, auch bei konstanter Dunkelheit nicht aufgeben. Sie behielten ihre Gewohnheit noch etwa eine Woche lang "pünktlich" bei. Für Rhythmen, die dem Tag- und Nachtwechsel angepasst sind und die nach dessen Ausschluss noch eine Zeitlang selbständig fortbestehen, besitzen wir noch einige interessante Beispiele.

So berichtet Massart (1893), dass die Cystoflagellate *Noctiluca miliaris*, die die häufige Ursache des sogenannten "Meeresleuchtens" ist, nachts heller leuchtet wie tags. Auch unter konstanten Bedingungen im Aquarium behielten die Protozoen diesen Rhythmus bei, wobei noch dahingestellt bleiben muss, worauf dieses Zeitgedächtnis beruht.

Über das Zeitgedächtnis des Leuchtkäfers *Luciola sinensis* berichtet Plate (1916). Die Käfer beginnen gewöhnlich abends gegen 19 Uhr zu leuchten. Plate brachte mehrere Glasröhrchen, in denen sich je ein Käfer befand, vormittags gegen 11 Uhr in eine Dunkelkammer. Die Tiere leuchteten den ganzen Tag über nicht, doch leuchteten sie abends auf, sobald im Freien die ersten Käfer sichtbar wurden. Selbst Tiere, die sich bereits seit zwei Tagen im Dauerdunkel befanden, fingen erst abends gegen 19 Uhr zu leuchten an. Plate sieht in diesem Zeitgedächtnis möglicherweise einen Hungerrhythmus. Hier mussen noch weitere Versuche Klarheit bringen.

Einen mit dem Wechsel von Tag und Nacht zusammenfallenden Farbwechsel zeigen die Stabheuschrecken (*Dixippus morosus*). Sie sind tags hell, nachts dunkel. Dieser Rhythmus erhält sich nach Schleip (1910, 1915) in konstanter Dunkelheit noch einige Wochen lang, danach tritt dauernde Dunkelstellung des Pigmentes ein, die aber schon bei kurzer Belichtung wieder dem normalen Farbwechsel Platz macht. Von besonderem Interesse ist die Tatsache, dass dieser Rhythmus umkehrbar ist: Schleip konnte zeigen, dass die Stabheuschrecken auch einem dem Wechsel von Tag und Nacht entgegengesetzten Beleuchtungswechsel folgen. Sie beantworteten ihn durch einen den natürlichen Bedingungen entgegengesetzten Farbwechsel, und behielten ihn sogar in dauernder Dunkelheit noch eine Zeitlang bei. Schleip erblickt in dieser Erscheinung eine Stoffwechselperiodik, die den Farbenwechsel als Begleit- bezw. Folgeerscheinung hat. Vielleicht kann man sich auch denken, dass der periodische Wechsel von Tag und Nacht, beziehungsweise hell und dunkel, eine erblich oder individuell erworbene Tendenz ausgebildet hat, alle 12 Stunden die Farbe zu wechseln. Das langsame Erlöschen des Rhythmus

unter konstanten Bedingungen spricht für eine "Erinnerung" des Organismus, die beim Fehlen des periodischen Reizes allmählich "vergessen" wird. Es wäre von Interesse, ob Versuche, einen anderen als den 12-stündigen Rhythmus zu induzieren, Erfolg hätten. Pauli (1926), dem die Umkehr des dem Tag- und Nachtwechsel angepassten Farbwechsels bei den Larven von *Salamandra* gelungen ist, misslangen Versuche, einen anderen als den 12 : 12-stündigen Rhythmus zu induzieren.

(4) *Jahreszeiten.*

Auf diesem Gebiete liegen mannigfache populäre Beobachtungen vor. So kennen wir z. B. das Fortziehen der Zugvögel beim Beginn der kalten Jahreszeit, und ihre Rückkehr aus den warmen Ländern, sobald es bei uns Frühling geworden ist. Welcher "Sinn" zeigt ihnen die richtige Zeit zur Rückkehr an? Es wird vermutet, dass wir es hier mit einer innersekretorischen "Uhr" zu tun haben. Nach den Untersuchungen von Milne Edwards (aus Pieron, 1910) zeigen gefangene Nachtigallen (*Luscinia*) bei Beginn des Winters auch dann noch deutlich den Trieb fortzuziehen, wenn sie in gleichmässig geheizten Treibhäusern gehalten werden. Dort können sie das Herannahen der Fröste unmöglich verspüren. Auch das Murmeltier (*Arctomys*) zeigt einen ähnlichen "Jahreszeitsinn," wie Pieron (1910) berichtet. Bekanntlich schläft es bei den ersten Frösten ein, um erst im Frühling seine volle Aktivität wieder zu gewinnen. Wird es an einem dauernd gut gewärmten Ort bei Nahrungsüberfluss gehalten, so zeigt es dennoch zu Beginn der kalten Jahreszeit das deutliche Verlangen einzuschlafen. Dieser "Zeitsinn" ist jedoch modifizierbar: wenn sich gleichsam gegen die Erwartung des Tieres im Versuchsraume Wärme und Nahrungsmenge weiterhin vorfinden, so entscheidet sich *Arctomys* ohne Mühe für das Aufgeben des geplanten Winterschlafes. Wie ganz allgemein das Problem des "Zeitgedächtnisses" noch vieler Untersuchungen bedarf, so ist es im besonderen erwünscht, dass über das Problem des "Jahreszeitsinnes" weitere Versuche eine Klärung schaffen mögen.

Bei den in diesem ersten Abschnitt berichteten biologischen Rhythmen traten nach dem Ausschalten der sie wahrscheinlich bedingenden periodischen Aussenreize noch eine Zeitlang rhythmische Lebenserscheinungen auf, um dann langsam zu erlöschen. In keinem Falle ist entschieden, ob dieses Zeitgedächtnis des Organismus ererbt oder individuell erworben ist, ob äussere oder innere Faktoren ausschliesslich, oder ein Zusammenwirken beider dabei die entscheidende Rolle spielen. Doch ist ihnen gemeinsam die Unmöglichkeit, dieses Zeitgedächtnis beliebig zu variieren, soweit darüber Untersuchungen angestellt sind. In den meisten Fällen handelt es sich um Beobachtungen, die noch der genauen Untersuchung bedürfen, sollen sie endgültig in das Kapitel Zeitgedächtnis aufgenommen werden. Es liegen vorerst nur bei sozialen Insekten exakte Untersuchungen vor, die von so grundlegender Bedeutung für das Problem des Zeitgedächtnisses sind, und es so wesentlich gefördert haben, dass ihnen ein eigener Abschnitt gewidmet werden muss.

III. EXPERIMENTELLE UNTERSUCHUNGEN ÜBER DAS ZEITGEDÄCHTNIS.

(1) *Das Zeitgedächtnis der Bienen.*

Exakte Untersuchungen mit Bienen haben überraschender Weise das Vorhandensein eines so ausgeprägten Zeitgedächtnisses bewiesen, dass hier grundlegende Unterschiede mit den bisher geschilderten Beispielen festzustellen sind. Es ist den Bienen im Rahmen des 24-Stundentages ohne weiteres möglich, sich beliebige Stunden zu merken; sie vermögen also anzugeben "wieviel Uhr" es ist. Das Zeitgedächtnis der Bienen stellt gleichsam eine höhere Stufe der bisher geschilderten "Biologischen Rhythmen" dar, auf dem Wege zu einem "Zeitgefühl" und bewussteren "Zeitschätzungsvermögen."

Schon Forel (1910) beobachtete, dass Honigbienen (*Apis mellifica*), die an einem Orte Honig oder Zuckerwasser gefunden hatten, dort am nächsten Tage wiederum zur selben Zeit Nachschau hielten. Dies legt schon die Vermutung nahe, dass die Bienen sich eine beliebige Tagesstunde einprägen können. Eine gelegentliche Beobachtung v. Buttel-Reepens (1915) lässt diese Fähigkeit berechtigt erscheinen: wie er feststellen konnte, wird der Buchweizen (Buckwheat) täglich nur bis gegen 10 Uhr vormittags von Bienen beflogen. Dies ist die Zeit seiner Nektarproduktion. Später am Tage sah v. Buttel-Reepen keine Bienen mehr auf den noch immer blühenden und duftenden Feldern. Falls nicht durch einzelne Kundschafter die Blüten von Zeit zu Zeit kontrolliert werden, kann nur ein ausgeprägtes Zeitgedächtnis den Bienen die Stunden erfolgreicher Tracht vermitteln.

Exakte Untersuchungen von Beling (1929) und Wahl (1932) haben hier eingesetzt. Zunächst konnte von Beling nachgewiesen werden, dass die Bienen tatsächlich ein präzises Zeitgedächtnis besitzen, das sie befähigt, sich jede beliebige Tagesstunde zu merken. Der Nachweis wurde durch die "Dressurmethode" erbracht, wie sie durch v. Frisch (1921) bei Bienen zu sinnesphysiologischen Untersuchungen als die ideale Methode eingeführt worden ist. An einem künstlichen Futterplatz werden Bienen zunächst durchgehend, mit Zuckerwasser oder verdünntem Honig, gefüttert. Während die Bienen sich vollsaugen, können sie leicht mit Markierungen versehen werden (v. Frisch, 1921). Unmarkierte Bienen werden getötet, sobald sie, durch die Verständigungstänze im Stock alarmiert, am Futterplatze erscheinen. So ist es möglich, das Verhalten einer ganz bestimmten Gruppe von Bienen, davon man jede einzelne durch die Markierung wohl unterscheiden kann, tagelang zu beobachten. Solche Gruppen von Bienen wurden von Beling täglich zu ganz bestimmten Tagesstunden gefüttert, zu den übrigen Stunden fanden sie niemals Futter am Dressurplatze vor. Die Anordnung des Futterplatzes blieb während aller Tagesstunden dieselbe, so dass es den Bienen nicht möglich war, durch optische oder sonstige Eindrücke die Zeit der Fütterung zu erkennen. Nur eine gründliche Nachschau am Futterplatz durch Niederlassen ermöglichte den Bienen die Feststellung, ob es dort Futter gab. Nach Ablauf einer Reihe von "Dressurtagen" wurde von Beling der "Versuchstag" abgehalten, eine Probe, ob die "Dressur-

zeit" bereits von den Bienen gelernt wurde. An diesem Tag wurde der Futterplatz den ganzen Tag über ununterbrochen beobachtet, *ohne dass Futter gereicht wurde.* Der Besuch jeder Biene wurde notiert. Es zeigte sich, dass der weitaus stärkste Bienenbesuch in die Zeit der gewohnten Futterstunden fiel. In die übrigen Stunden fielen nur wenige Besuche am Futterplatz, und diese fast ausschliesslich in die erste Stunde vor Beginn der "Dressurzeit."

Beling gelangen Dressuren auf jede beliebige Tageszeit. Sie konnte weiter zeigen, dass auch Dressuren auf zwei und drei Tageszeiten an *einem* Tage möglich sind. Die Zwischenpausen dürfen bei solchen Dressuren nicht zu kurz sein, sondern müssen nach Wahl mindestens 2 Stunden betragen. Bei kürzeren Zwischen-pausen verwischen sich die Besuchsmaxima und die Bienen suchen den ganzen Tag über am Futterplatze nach. In den Monaten der längsten Tage gelang Wahl eine Dressur ein und derselben Bienenschar auf fünf Tageszeiten. Dabei war der Futterplatz stets derselbe. Wahl fütterte in einem anderen Versuch ein und dieselbe Schar vormittags an Platz A und nachmittags an Platz B. Dies gelang mit der Einschränkung, dass die Bienen am Beobachtungstage, wenn sie zur richtigen Zeit vergeblich bei Platz A nachgesucht hatten, auch bei Platz B Nachschau hielten, und ebenso nachmittags, sodass im Ganzen vier Besuchsmaxima zu verzeichnen waren: zwei bei A vor- und nachmittags, und zwei bei B vor- und nachmittags. Offenbar entspricht es den Bienen—wohl wegen ihrer bekannten "Blumenstetigkeit" (vergl. Anmerkung S. 27)—nicht, verschiedene Futterplätze täglich zu verschiedenen Zeiten zu befliegen. Sie identifizierten anscheinend in Wahls Versuch die beiden Futterplätze, als ob es sich um die Nektarquellen ein und derselben Blumenart zu zwei verschiedenen Tageszeiten handelte. Darauf wird später noch bei der Frage nach der biologischen Bedeutung des Zeitgedächt-nisses eingegangen werden.

Wahl konnte nachweisen, dass das Zeitgedächtnis der Bienen starr an den 24-Stundenrhythmus gebunden ist. Weder Wahl noch Beling gelangen Dressuren auf andere Zeitintervalle. Bei den Dressuren auf mehrere Tageszeiten handelt es sich nur scheinbar um einen Widerspruch, denn auch hier erfolgen die Fütterungen zu bestimmten, alle 24 Stunden wiederkehrenden Zeitpunkten. Bei Dressuren dagegen, die z. B. in einem 19-stündigen Rhythmus erfolgten, wobei das Intervall stets dasselbe, die Tagesstunde aber stets eine andere ist, zeigte sich völliges Versagen der Bienen. Ihre Tendenz ging, wie am Versuchstage offenbar wurde, dahin, 24 Stunden nach der letzten Fütterung zu erscheinen. Dies ist der Beweis, dass ein anderer Rhyth-mus als der 24-stündige den Bienen nicht adäquat ist.

Dressuren auf "Zeit" gelangen auch zu jeder beliebigen *Nachtstunde* (Beling, 1929). Dieser Nachweis konnte erbracht werden, durch Verbringen eines ganzen Bienenvolkes in einen lichtabgeschlossenen und mittels elektrischer Beleuchtung konstant erhellten Raum. Auch hier erschienen die Bienen 24 Stunden nach der letzten Fütterung zahlreich am Dressurplatz, während in den übrigen Stunden der 24-stündigen Beobachtungsdauer die Frequenz am Futterschälchen gering war oder ganz aussetzte. Das Zeitgedächtnis der Bienen ist danach nicht an den Wechsel von Tag und Nacht gebunden, sondern unabhängig von den natürlichen Lichtver-

hältnissen. Man kann es mit einer Wecker-Uhr vergleichen, die nach 24 Stunden läutet, gleichgültig welche Tagesstunde als Ausgangspunkt eingestellt wird.

Die Untersuchungen von Wahl haben gezeigt, dass Dressuren auf eine bestimmte Tageszeit noch etwa eine Woche lang im Gedächtnis der Biene haften. Dies ist insofern von Interesse, als es beweist, dass die Bienen sich auch nach einer Reihe von Schlechtwettertagen noch an die gewohnten Futterstunden erinnern können. Dadurch gewinnt die Beobachtung v. Buttel-Reepens, dass die Bienen nur vormittags auf den Buchweizenfeldern zu sehen sind (das sind die Stunden, in denen der Buchweizen Nektar produziert), neue Bedeutung. Die Bienen können also diese Stunden auch dann im Gedächtnis behalten, wenn ihnen der Flug auf die Buchweizenfelder durch Regen einige Tage unmöglich gemacht wird. Es ist dann nicht nötig, den Buchweizen als Futterquelle neu zu entdecken, und durch viele vergebliche Flüge—wie zu Beginn einer "Zeitdressur"—die Stunden ergiebiger Tracht herauszufinden. Hier liegt vielleicht die von verschiedenen Forschern vermutete biologische Bedeutung des Zeitgedächtnisses. Es gibt, wie bereits bekannt ist, ausser dem Buchweizen noch eine ganze Anzahl von Bienennährpflanzen, die nur zu bestimmten Tagesstunden Nektar oder Pollen in reichlicher Menge produzieren. Es ist anzunehmen, dass Bienen, die an solchen Blumen sammeln, sich ebenso wie bei den "Zeitdressuren" diese Stunden einprägen. Dies wird wahrscheinlich durch weitere Beobachtungen. Beutler (1930) konnte feststellen, dass auch bei Pflanzen, die den ganzen Tag über Nektar bezw. Pollen produzieren, ein tägliches Produktionsmaximum zu bestimmter Stunde eintritt. Es liegt nahe, anzunehmen, dass die Honigbienen diese Stunden des Maximalproduzierens wahrnehmen, und die Pflanzen während dieser Tageszeiten am häufigsten befliegen. Tatsächlich gelangen Wahl (1933) in Nachahmung dieser Verhältnisse Dressuren von Honigbienen auf zeitliche Konzentrationsunterschiede des am kunstlichen Futterplatze gebotenen Zuckerwassers. Am Versuchstage, an dem ohne Futterdarbietung lediglich beobachtet wurde, erschienen am Futterplatz, wo bisher jeweils den ganzen Tag über durchgehend gefüttert worden war, zu *denjenigen* Stunden die meisten Bienen, zu welchen während der Dressur das Zuckerwasser am konzentriertesten geboten worden war. Diese Beobachtungen stehen in schönem Einklang mit dem Bestehen eines so gut ausgebildeten Zeitgedächtnisses und seiner Beweglichkeit im Rahmen des 24-stündigen Tages. Im Gegensatz zu Wahl und Beling, die daraus auf eine biologische Bedeutung des Zeitgedächtnisses schliessen, kommt Lutz (1934) auf Grund seiner Untersuchungen zu dem Ergebnis, dass eine solche nicht besteht. Er schloss folgendermassen: Wenn die Bienen ihren "Zeitsinn" dazu benützen, um sich zu einer Tageszeit zu dieser Blütenart, zu einer anderen Stunde zu jener Spezies leiten zu lassen, dann müssen sie dazu imstande sein, die Kombination von mehreren Farben, Düften, Zeiten und Futter zu assoziieren[1]. Er konstruierte einen Apparat, in dem die Bienen zu

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[1] Hier liegt jedoch bereits ein Trugschluss. Es ist nicht der Fall, dass die Honigbiene täglich verschiedene Blumenarten befliegt. Sie ist "blumenstet," das heisst, sie befliegt eine bestimmte Blumenart bis zu deren Verblühen. Hierauf beruht der Nutzen der Biene als Bestäuberin der Blüten. Flöge sie von einer Spezies zu einer anderen Spezies, so würden ja Samen der einen Art nicht wieder zu Individuen derselben Art gelangen können. Es ist also nicht der Fall, dass, wie Lutz annimmt, die Biene im Lauf eines Tages Blumen verschiedener Farben und Düfte befliegt. Auf diese Verhältnisse wird oben weiter eingegangen.

verschiedenen Zeiten Futter vorfanden, zu anderen Stunden war es ihnen nicht
zugänglich, sondern durch ein Drahtgitter abgeschlossen. Es wurde in zwei
Kästchen jeweils vormittags zwei Stunden lang in dem einen Kästchen bei bestimm-
ter Dressurfarbe gefüttert, nachmittags in dem anderen wiederum zwei Stunden bei
einer anderen Farbe. Um den Versuch den natürlichen Bedingungen anzugleichen,
blieb die Versuchsanordnung stets dieselbe, auch das Zuckerwasser blieb in den
Dressurkästchen stets aufgestellt stehen. Lediglich durch die Drahtgitter wurden
die Futterstunden reguliert. Die Gitter befanden sich im Inneren der Dressur-
kästen, konnten also von den anfliegenden Bienen nicht bemerkt werden. Die
Bienen flogen an, krochen durch die in der Mitte der Farbenschablone in das
Kästchen führende Öffnung, und gelangten bei geöffnetem Gitter von hier aus in
die "Futterkammer." Lutz erfand ein sehr sinnreiches "Einbahnstrassensystem,"
das die Bienen zwang, das Kästchen an einem besonderen Ausgang zu verlassen,
sodass die nachkommenden Bienen nicht durch die umkehrenden Bienen bereits
verständigt werden konnten, ob es im Kästchen etwas zu finden gab oder nicht. Die
Versuchsbienen waren markiert. Lutz veränderte nun nach wochenlanger Dressur
auf zwei Tageszeiten und zwei Farben eines Tages willkürlich die Versuchsanord-
nung. So wurden z. B. morgens die Gitter zur Futterzeit nicht geöffnet, und blieben
auch während der gewohnten Nachmittagszeit geschlossen. Dagegen wurde nach
dem Termin der gewohnten Nachmittagsfütterung "unerwartet" das Gitter
geöffnet. Eine einzelne Biene fand dies, und sofort war die ganze Schar alarmiert
und beutete eifrigst die neuentdeckte Gelegenheit aus.

In einem anderen Versuch wurden die Bienen nach längerer Dressur auf die
Vor- und Nachmittagszeit (zugleich mit einer Vor- und Nachmittagsfarbe) plötzlich
den ganzen Tag über gefüttert, und zwar nur auf der "Vormittagsseite" und
"Vormittagsfarbe" des Apparates. Die "Nachmittagsseite" blieb den ganzen Tag
über geschlossen. Die Bienen beflogen die "Vormittagsfarbe," bei der es den
ganzen Tag über etwas zu finden gab, zu 90 %, bis zum Abend. Zur "rich-
tigen" Nachmittagszeit wurde zwar auch von wenigen Bienen vergebliche
Nachschau gehalten, doch konzentrierte sich der Besuch deutlich von morgens an
auf die "Vormittagsfutterquelle." Lutz zieht daraus die folgenden Schlüsse:
Die Bienen haben zwar eine bestimmte Periodizität erlernt, aber es hat sich
daraus nicht die feste Gewohnheit herausgebildet, während der Morgenstunden
die eine Farbe, während der Nachmittagsstunden die andere Farbe zu befliegen.
Wenn bei der einen Farbe vormittags, bei der anderen Farbe nachmittags gefüttert
wird, so kommen sie in grosser Anzahl zur richtigen Farbe in der richtigen Zeit,
aber daneben suchen immer noch einzelne Spürbienen bei der anderen Farbe zur
"falschen" Zeit nach. Findet sich dann "wider Erwarten" dort auch Futter vor,
so passen sich die Bienen trotz der Dressur sogleich der neuen Situation an. Lutz
ist nach seinen Untersuchungen überzeugt, dass die Bienen unter natürlichen
Umständen nicht von ihrem Zeitgedächtnis Gebrauch machen, sondern sich
lediglich auf die Alarmierung durch die Spürbienen verlassen. Er schliesst sein
Urteil in den folgenden Worten zusammen (S. 9): "During daylight hours there are
always 'prospecting' bees hunting here and there in the buckwheat fields as well as

elsewhere. If they find buckwheat offering nectar they feed and return to the hive. Their return starts an activity in the swarm and soon the buckwheat field is full of bees. When the buckwheat stops offering nectar most of the bees stop visiting it and turn their attention elsewhere." Über das Zeitgedächtnis und seine Anwendung in der freien Natur ist Lutz folgender Ansicht (S. 10): "Though the bees may be able to acquire the complicated associations involved in Beling's solution of the 'buckwheat problem' they do not need and probably do not use this ability. They take their food where and when they find it, apparently being guided largely by the circumstances of the moment."

Über die mögliche biologische Bedeutung des Zeitgedächtnisses, die von Wahl (1932) auf Grund besonderer Untersuchungen angenommen wird, bestehen demnach verschiedene Ansichten, die noch geklärt werden müssen. Bei den Versuchen von Lutz könnte der Einwand gemacht werden, dass die Bienen den ganzen Versuchsapparat als solchen für *eine* Futterquelle gehalten haben, die morgens und nachmittags "honigte," wie bei den Dressuren auf mehrere Futterzeiten. Dabei brauchten die verschiedenen Eingänge und Farben die Bienen nicht zu verwirren. So ist es erklärlich, warum die Bienen stets bei *beiden* Kästchen Nachschau gehalten haben, da sie annahmen, dass ihre "Blume" zweimal am Tage Futter bot. Hierzu im Einklang stehen die bekannten Tatsachen, dass Dressuren auf mehrere Tageszeiten am selben Futterplatz ohne weiteres gelingen (Beling und Wahl). Es gelingt dagegen nur ganz unvollkommen, ein und dieselbe Schar an zwei verschiedene Orte zu zwei verschiedenen Zeiten zu gewöhnen. Die Bienen suchen am Versuchstage an beiden Orten zu beiden Zeiten nach, lediglich ist das Maximum des Besuches am "richtigen" Ort zur "richtigen" Zeit etwas höher. Vielleicht spricht hier die bekannte "Blumenstetigkeit" der Honigbiene mit, und ist von Lutz in seinen Schlussfolgerungen nicht genügend berücksichtigt worden. Die Tatsache, dass die Bienen nicht lernen, an einem Ort nur vormittags und am anderen Orte nur nachmittags zu verkehren, sondern am Versuchstage an beiden Orten zu beiden Zeiten Nachschau halten, spricht für das Bestehen einer Blumen- und Zeitstetigkeit. Diese schliesst nicht aus, dass einzelne Spürbienen diese Schar auch alarmieren können, wenn die besuchte Futterquelle unerwartet zu anderer Zeit fliesst. Dass bei unregelmässiger Fütterung die Bienen *dauernd* Nachschau am Futterplatze halten, während diese bei regelmässiger Fütterung nahezu aufhört, beobachtete auch v. Dobkiewicz (1912).

Nach der Meinung des Verfassers ist gerade die von Lutz gemachte Feststellung, dass Bienen sich nicht auf verschiedene Orte und Zeiten dressieren lassen, ein Beweis gegen die Schlussfolgerung von Lutz: "When the buckwheat stops offering nectar most of the bees stop visiting it and turn their attention elsewhere." Dass, wie Lutz wohl irrtümlich annimmt, die Bienen täglich mehrere Blumenarten besuchen, ist zumindest während der Zeit des Hochsommers nicht der Fall. Die "Blumenstetigkeit" lässt erst in den Herbstmonaten nach, wenn die sommerlichen Nektarquellen langsam versiegen.

Der Sitz des Zeitgedächtnisses der Bienen. Die Frage, wo sich die "Uhr" befindet, die den Bienen im Stock so genau die Stunden reichlichen Futters angibt,

hat sich bereits Beling in einer Anzahl von Versuchen gestellt. Zunächst ist man geneigt, einen Hungerrhythmus anzunehmen. Diese Schlussfolgerung liegt nahe, da ja die Zeitdressur in Gestalt einer Fütterung zu bestimmter Stunde erfolgt. Bei näherer Betrachtung aber wird diese Annahme unwahrscheinlich. Sie erklärt z. B. nicht, wie es den Bienen möglich ist, sich nicht nur den Beginn, sondern auch das Ende der Dressurzeiten zu merken. Das Nachlassen der Besuche am Futterplatz ist von allen Beobachtern an den Versuchstagen stets festgestellt worden, wenn die Dressurzeit abgelaufen war. Es ist natürlich selbstverständlich, dass am Versuchstage kein Futter geboten wird. Auch die Zeitdressuren auf mehrere Tageszeiten können nicht mit einem Hungerrhythmus erklärt werden, denn es ist nicht anzunehmen, dass Bienen sich ihr Hungergefühl je nach der Dressur in " Raten " von 1–5 einteilen können. Wahl (1932) konnte überdies nachweisen, dass auch zeitdressierte Bienen zu allen Tageszeiten innerhalb des Stockes Nahrung aufnehmen. Ausserdem gelangen ihm ohne weiteres Zeitdressuren auf Pollen, der von Bienen ja bekanntlich in Form von "Höschen" eingetragen wird. Die eingehenden Untersuchungen Wahls sprechen klar gegen die Annahme einer "Hungeruhr."

Auch die uns bekannten tagesperiodischen Aussenfaktoren kommen als Uhr nicht in Frage. Die Vermutung, dass der Stand der Sonne den Bienen die Tageszeit angibt, konnte bereits durch die erfolgreichen Dressuren auf bestimmte Stunden in einem vom Tageslicht vollkommen abgeschlossenen Raum abgelehnt werden. In diesem Versuchsraum, in dem die Bienen nicht durch die Nachtkühle und die Dunkelheit am Ausfliegen verhindert waren, wie dies im Freien nachts der Fall ist, gelangen Dressuren auf jede beliebige Nachtzeit. (Der Versuchsraum war konstant erleuchtet.) Auch in der Dunkelkammer gelangen Dressuren auf mehrere Tageszeiten.

Der Nachweis, dass die Bienen sich nicht an den Schwankungen der Aussentemperatur, die überdies nicht gleichmässig sind, die Zeit merken, konnte von Wahl und Beling ebenfalls erbracht werden. Die Luftfeuchtigkeit gibt nach Beling's (1929) Untersuchungen ebenfalls den Bienen keine zeitlichen Anhaltspunkte. Der tagesperiodisch verlaufende Gang der elektrischen Leitfähigkeit der Atmosphäre, der auch im geschlossenen Versuchsraum noch wirksam ist, wurde von Beling (1929) durch das Aufstellen eines Radiumpräparates während des Beobachtungstages unwirksam gemacht. Die Bienen kamen trotzdem zur richtigen Zeit zum Futterplatz. Die Annahme Beling's, dass ein uns noch unbekannter tagesperiodischer Aussenfaktor der Zeitmesser für die Bienen sein könne, hat Wahl (1932) zu neuen Untersuchungen veranlasst. Er zog in Betracht, dass die sogenannte "Höhen- oder Raumstrahlung" in ihrem Gang die "Uhr" sei, an der die Bienen die Zeit abzulesen vermögen. Diese Strahlung ist durchdringender als die härtesten bekannten Gamma-Strahlen und war deshalb bei dem Luftionisationsversuch Beling's durch das Aufstellen eines Radiumpräparates nicht ausgeschaltet worden. Deshalb stellte Wahl Untersuchungen in einem Steinsalzbergwerk (bei Kochendorf a. N.) an. Durch die abschirmende Wirkung des Steinsalzes konnten hier die in das Bergwerk verbrachten Bienenvölker ohne die Einwirkung einer tagesperiodischen Luftionisation auf Zeit dressiert werden. Von besonderem

Interesse ist es, dass Wahl seine Versuche in demselben Bergwerk anstellte, in dem auch Cremer's (1923) Untersuchungen über die tagesperiodischen Schlafbewegungen der *Phaseolus*-Blätter ausgeführt wurden. Cremer fand, dass die Blätter von im Bergwerk kultivierten *Phaseolus*-Pflanzen keine rhythmischen Schlafbewegungen ausführten. Wenn die Pflanzen jedoch in ein verdunkeltes Zimmer an die Erdoberfläche verbracht wurden, so stellten sich die tagesperiodischen Schlafbewegungen sogleich ein. Cremer nimmt zur Erklärung dieser Erscheinung einen uns noch unbekannten tagesperiodischen Faktor an, der nur an der Erdoberfläche wirksam ist und die Schlafbewegungen der Pflanzen reguliert. Die Vermutung lag nahe, dass dieser Faktor auch für das Zeitgedächtnis der Bienen verantwortlich zu machen ist. Doch bestätigte sich diese Vermutung nicht. Es gelangen Wahl mit Bienen in diesem Bergwerk (in 180 m. Tiefe) ebenfalls Dressuren auf beliebige Tageszeiten!

Wie Wahl zeigen konnte, ist das Zeitgedächtnis keine im Einzelleben der Biene erworbene Fähigkeit. Es lassen sich auch Bienen erfolgreich "auf Zeit" dressieren, die den Tag- und Nachtwechsel nie kennen lernten. Zu diesem Nachweis stellte Wahl (1932) ein ganzes Volk aus Bienen zusammen, die, einschliesslich der Königin, aus Brutwaben in einem Thermostaten zum Schlüpfen kamen. Keines der annähernd tausend Individuen hatte den normalen Stockbetrieb erlebt und war jemals mit älteren, "erfahrenen" Bienen in Berührung gekommen. Dennoch liessen sie sich ohne weiteres auf Zeit dressieren.

Eine ganz entscheidende Förderung des Problems verdanken wir Grabensberger (1934). Ihm ist es gelungen, nachzuweisen, dass wir das Zeitgedächtnis der Bienen nicht in Beziehung zu äusseren, sondern zu *inneren* Faktoren zu bringen haben. Nach seinen Untersuchungen an Ameisen, Bienen, Wespen und Termiten beruht das Zeitgedächtnis auf dem Rhythmus des Stoffwechsels, und zwar des Zellstoffwechsels. Den Beweis, dass die Bienen an der Geschwindigkeit des Stoffumsatzes im Körper die Zeit ablesen, erbrachte er auf klassische Weise: er verfütterte an eine Schar zeitdressierter Bienen am Tage vor der Beobachtung Stoffe, die die Umsatzgeschwindigkeit hemmen beziehungsweise befördern. Die Beimischung des umsatzherabsetzenden Euchinins in das gebotene Zuckerwasser hatte zur Folge, dass die Bienen am nächsten Tage verspätet zur Futterstelle kamen. Entsprechende Versuche mit dem die Geschwindigkeit des Stoffumsatzes fördernden Jodthyreoglobulin bewirkten ein Zufrühkommen der Bienen an den Futterplatz. Damit ist das interessante Problem bereits grösstenteils geklärt. Die Untersuchungen von Kalmus (1934) stehen im Einklang mit Grabensberger's Untersuchungen. Auch dieser Forscher erzielte durch die Einwirkung von Euchinin ein Zuspätkommen der Bienen. Das Gleiche war der Fall, wenn er die Bienen der Einwirkung von Kälte, die ja ebenfalls den Stoffwechselumsatz zu hemmen vermag, aussetzte. Versuche mit Bienen, die starker Erwärmung ausgesetzt wurden, deuten an, dass durch diese Weise künstlicher Beschleunigung des Stoffumsatzes eine entsprechende Verfrühung in dem Erscheinen am Futterplatz herbeigeführt werden kann, ebenso wie sie Grabensberger mit der Verfütterung von Jodthyreoglobulin erhielt. Doch fällt es auf, dass geringe Temperaturerhöhung von etwa 10 Grad, ebenso wie bei den Versuchen von Wahl, noch keinen Einfluss auf die Bienen ausübt. Sowohl Grabens-

berger wie auch Kalmus nehmen an, dass die Biene befähigt ist, Temperatur-
einwirkungen weitgehend zu kompensieren. Beeinflussungen des Zeitgedächt-
nisses auf diese Weise gelingen daher nach der Ansicht von Kalmus nur dann,
wenn mit ganz extrem hohen Temperaturen (39–41° C.) oder extremer Abkühlung
bis zur Kältestarre (5–7° C.) gearbeitet wird.

Die Versuche von Kalmus, durch Verfüttern von pulverisierter Thyreoidea den
Besuch am Futterplatz zeitlich zu verschieben, wie dies Grabensberger mit Jod-
thyreoglobulin gelungen ist, misslangen.

Wir verdanken Grabensberger die Feststellung, dass auch andere soziale Insekten
ein Zeitgedächtnis besitzen. Über seine Untersuchungen, die die an Bienen und
Wespen erbrachten Nachweise über die Natur des Zeitgedächtnisses sehr wesent-
lich gefördert haben und sie weitgehend befestigen konnten, soll im Nachfolgenden
berichtet werden.

(2) Untersuchungen über das Zeitgedächtnis der Ameisen.

Die Untersuchungen Grabensbergers (1933) mit Ameisen—als den Bienen in
vieler Hinsicht gleichgearteten Insekten—ergaben das Bestehen eines Zeitgedächt-
nisses, das von dem der Bienen durch seine rhythmische Modifizierbarkeit unter-
schieden ist. Grabensberger arbeitete mit Individuen von Myrmica-Arten, Lasius-
Arten und dem nicht blütenbesuchenden Camponotus ligniperdus. Er entnahm die
Tiere Freilandkolonien in einer Anzahl von je nach Bedarf 50-4000 Stück und
verbrachte sie in die Beobachtungsnester. Diese bestanden aus einem Hauptnest-
raum, der durch ein Verbindungsstück auf zwei Seiten mit je einem Dressurraum
in Verbindung stand. Dieser war in mehrere miteinander verbundene Räume
geteilt. Eine alle 5 Minuten vorgenommene Zählung aller im Dressurraum an-
wesenden Tiere ergab die Frequenzzahl. Die Dressur bestand, wie bei den Bienen,
in Fütterungen und zwar von Zuckerwasser, Honig und gelegentlich Fleisch. Da die
Ameisen keinen "Gemeinschaftsmagen" wie die Bienen besitzen, also die auf-
genommene Nahrung bei sich behalten und nur gelegentlich an andere Individuen
weitergeben, gestaltete sich die Dressur schwieriger als bei der Honigbiene. Es
musste darauf geachtet werden, dass die Versuchstiere nicht zu bald überfüttert
waren, da sie dann nicht mehr zur Futterstelle kamen. Als besonders auffallend ist
die Feststellung zu erwähnen, dass Ameisen bereits nach zwei- bis dreimaliger
Fütterung eine Zeitdressur "begriffen" hatten. Die Ameisen liessen sich bei der
geschilderten Versuchsanordnung auf jede beliebige Tageszeit dressieren. Auch
auf mehrere—bis zu 5—Tageszeiten sind sie dressierbar. Überraschend an Grabens-
berger's Ergebnissen ist vor allem, dass die Ameisen sich im Gegensatz zu den Bienen
auf beliebige Rhythmen dressieren lassen. Es gelangen Versuche im 3-, 5-, 21-, 22-,
26- und 27-stündigen Rhythmus. Dabei zeigten sich interessante Unterschiede
zwischen den blütenbesuchenden und den nicht blütenbesuchenden Arten. Die
nicht blütenbesuchende Art Camponotus ligniperdus lernte in kürzester Zeit jeden
beliebigen Rhythmus, ohne eine Bevorzugung des 24-stündigen "biologischen
Rhythmus" erkennen zu lassen. Bei der blütenbesuchenden Art Myrmica rubida
laevinodis zeigte sich dagegen, wie bei den Bienen, deutlich die Tendenz, 24 Stunden

nach einer Fütterung wieder im Dressurraum Nachschau zu halten. Doch liess auch diese Art sich auf andere beliebige Rhythmen dressieren, im Gegensatz zu den Bienen, die unabänderlich an den 24-stündigen Rhythmus gebunden sind. Hier bestehen grundlegende Unterschiede. Sie lassen sich möglicherweise aus der völligen Abhängigkeit der Bienen von den blütenbiologischen Verhältnissen erklären, die ja für die Ameisen nicht in diesem Ausmasse besteht.

Es gelangen Grabensberger bei Ameisen sogar Dressuren auf gleichzeitig zwei verschiedene Rhythmen. So z. B. kam ein und dieselbe Ameisengruppe in 22- und 24-stündiger Periode zum Futterplatz. Die Frage nach dem Sitz des Zeitgedächtnisses bei den Ameisen kann in der gleichen Weise beantwortet werden wie bei den Bienen. Durch Regulierung der Umsatzgeschwindigkeit des Organismus erkannte er diese als die "Uhr," an der das Individuum die Zeit ablesen kann. Durch Verfütterung von Jodthyreoglobulin an zeitdressierte Tiere erzielte er eine Erhöhung der Umsatzgeschwindigkeit mit dem Erfolg, dass die Tiere früher zur Futterstelle kamen. Nach Verfütterung von Euchinin dagegen, das die Umsatzgeschwindigkeit herabsetzt, kamen zeitdressierte Tiere später zur Futterstelle. Grabensberger schloss weiter, dass eine Erhohung bezw. Erniedrigung der Nesttemperatur dieselbe Wirkung auf die Umsatzgeschwindigkeit des Organismus haben müsse, wie die verwendeten Mittel. Deshalb wurden die Nester zeitdressierter Tiere am Beobachtungstage einer Erhöhung bezw. Erniedrigung ihrer Temperatur unterworfen. Tatsächlich kamen die Ameisen im einen Falle früher, im anderen Falle später zur Futterstelle. Einige weitere Kontrollversuche Grabensbergers mit verschiedenen anderen Stoffen, die auf den Stoffwechsel einwirken (1934 *b*), bestätigen die früheren Versuchsergebnisse. Vor allem interessant sind seine Versuche mit Verfütterung von Salicylsäure, die bekanntlich den Umsatz beschleunigt. Die Versuche wurden wiederum mit *Myrmica* und *Lasius* durchgeführt. Die Tiere erhielten nach zweitägiger Fütterung zur selben Zeit am dritten Tag eine Mischung von 0·008 % Salicylsäure in das Futter. Das Besuchs-Maximum am nächsten Tag (Beobachtungstag) war um 3½ Stunden vorverschoben! Diese Wirkung der Salicylsäure hielt sogar noch am nächsten Tage vor, an dem die Ameisen wiederum genau 3½ Stunden zu früh an der Futterstelle erschienen. Eine Beigabe von 0·02 % Salicylsäure verursachte eine Verfrühung der Ameisen um 8 Stunden! Eine 2½-fache Dosis brachte also entsprechend eine etwas mehr als doppelte Wirkung hervor. Beimischungen von gelbem Phosphor und weissem Arsenik hatten dieselbe "verfrühende" Wirkung auf das Zeitgedächtnis— nämlich die Umsatzgeschwindigkeit des Stoffwechsels—wie Salicylsäure. Die Versuche mit den Stoffen der Arsenik-Gruppe stellte Grabensberger aus besonderen Erwägungen an, die in entsprechenden Versuchen auch ihre Bestätigung fanden. Er ging von den folgenden Tatsachen aus: Die Stoffe der Arsen-Gruppe haben beim Säugetier oxydationshemmende Wirkung. In kleinen Mengen dem Organismus zugeführt, fördern sie dadurch das Wachstum von Geweben, und rufen so eine Gewichtszunahme hervor. In grösseren Mengen zugeführt aber haben die Stoffe eine so starke oxydationshemmende Wirkung, dass infolge zu mangelhafter Oxydation der Aufbaustoffe Materialverlust, d. h. Abmagerung, eintritt. Grabens-

berger untersuchte die Wirkung des Arsens auf den Insektenorganismus mit folgender Voraussetzung: Bei einer sehr geringen Menge muss "Verspätung" am Futterplatz eintreten, da im Organismus Aufbau eintritt, der den allgemeinen Umsatz hemmt. Sodann muss eine *mittlere* Dosis wirkungslos bleiben, da bei ihr sich Abbau und Stoffansatz die Wage halten. Die Ameisen müssten bei dieser Dosis zur "richtigen" Zeit erscheinen. Und schliesslich müsste eine *starke* Dosis die Ameisen "verfrüht" zum Futterplatz führen, da durch sie ein der Abmagerung bei den Säugetieren entsprechender schnellerer Stoffumsatz hervorgerufen wird. Diese Schlussfolgerungen bestätigten sich in glänzender Weise: nach einer Dosis von 0·0001 % Arsenik kommen die Ameisen zur normalen Zeit, da diese Menge zunächst zu klein ist und wirkungslos bleibt—nach 0·0005 % um 3½ Stunden zu spät, nach 0·00075 % sind sie wieder zur richtigen Zeit anwesend und bei 0·001 verfrühen sie sich um 3½ Stunden. Entsprechend verfrühten sie sich bei einer Dosis von 0·002 % um 6½ Stunden. Die Untersuchungen Grabensberger's sind von grundlegender Bedeutung. Sie können zeigen, dass die Wirkung eines Stoffes, der beim Säugetier die Umsatzgeschwindigkeit beschleunigt oder verzögert, bei den Ameisen dieselbe ist. Mit Bienen wurden z. T. entsprechende Ergebnisse gewonnen (s. o.), mit Salicylsäure und den Stoffen der Arsen-Gruppe stehen Versuche bei ihnen noch aus. Über den Nachweis hinaus, dass das Zeitgedächtnis der Ameisen, wie bei den Bienen, in der Umsatzgeschwindigkeit des Stoffwechsels zu suchen ist, hat Grabensberger gefunden, dass die Ameisen auf geringste Dosen umsatzhemmender bezw. beschleunigender Substanzen ausserordentlich fein reagieren. Er empfiehlt daher die Ameise als das geeignete Versuchsobjekt zur Feststellung, welchen Einfluss irgendeine Substanz auf die Intensität des Stoffwechsels hat.

(3) *Untersuchungen über das Zeitgedächtnis der Termiten.*

Von Grabensberger (1933) wurden auch Termiten (*Termes lucifugus*) zur Untersuchung herangezogen, wenn auch noch nicht in sehr ausgedehntem Masse. Doch zeigte es sich hierbei bereits, dass die Termiten offenbar ebenfalls *ein* Zeitgedächtnis besitzen. Die "Dressur" bestand wie bei den Ameisen in einer zeitlich sich gleichmässig wiederholenden Fütterung. Als Futter wurde feuchter Mulm von Salweiden genommen, da die Termiten Honig, Mehl, Zucker und Fleisch ignorieren. Eine Dressur auf einen 21-stündigen Rhythmus gelang. Ob die Termiten zu dem 24-Stundenrhythmus in einer besonderen biologischen Beziehung stehen, wurde noch nicht untersucht.

(4) *Untersuchungen über das Zeitgedächtnis bei Fischen.*

Wie die vorliegenden Untersuchungen ergeben haben, besitzen soziale Insekten wie Bienen, Ameisen und Termiten ein Zeitgedächtnis. Daher ist es von besonderem Interesse zu erfahren, ob auch andere Tiergruppen in so ausgedehntem Masse damit begabt sind. Bisher liegen dafür ausser den als "Biologische Rhythmen" bezeich-

neten Beobachtungen noch keine genauen Untersuchungen vor. Sie sind bisher nur bei Fischen, mit negativem Erfolg, in Angriff genommen worden. Beling (1929) versuchte Ellritzen (*Phoxinus laevis*) auf eine bestimmte Futterstunde zu dressieren. Täglich wurden die geblendeten Tiere mittels Glasstab zur bestimmten Tageszeit gefüttert, wobei jedes Geräusch bei der Annäherung an das Aquarium sorgfältigst vermieden wurde. Trotz wochenlanger Dressurfütterung zeigten die Fische kein Anzeichen von Erregung, wenn die Futterstunde herannahte. Es ist zu bemerken, dass geblendete Fische ausgezeichnet imstande sind, deutliche Äusserungen ihrer Futtererwartung zu geben, wie die Versuche v. Frisch's (1923), Stetter's (1929), und v. Frisch und Stetter's (1932) mit *Phoxinus* hinreichend ergeben haben. Diese Anzeichen von Futtererwartung gaben dagegen "zeitdressierte" Ellritzen nur dann, wenn sie das an einem Glasstab gereichte Futter mit ihrem Geruchsinn wahrnahmen. An dem Beobachtungstag, an dem es kein Futter gab, verhielten sich die Fische den ganzen Tag über gleichmässig, und zeigten beim Herannahen der Futterstunde keinerlei Unruhe. Ebenso verhielten sich Ellritzen, die nicht geblendet waren, und in einer Dunkelkammer bei konstanter Beleuchtung wochenlang zur selben Stunde gefüttert wurden. Weitere Untersuchungen mit Wirbeltieren sind, soweit dem Verfasser bekannt ist, noch nicht angestellt worden.

IV. EXPERIMENTELLE UNTERSUCHUNGEN ÜBER DAS ABSCHÄTZEN KURZER ZEITSPANNEN.

Wie die Untersuchungen Beling's, Grabensberger's und Wahl's zeigen konnten, besitzen Bienen und Ameisen die Fähigkeit, rhythmische Zeitintervalle zu "erlernen" und beizubehalten, solange die rhythmischen Anstösse (gebotenes Futter) dauern. Bei den Bienen ist diese Fähigkeit an den biologischen 24-Stunden-rhythmus gebunden, ebenso, doch in schwächerem Masse, bei den blütenbesuchen-den Ameisen. Über die wahrscheinliche biologische Bedeutung dieser Tatsache liegen wichtige Untersuchungen und Beobachtungen vor (Beutler, 1930; Wahl, 1933). Bei den nicht blütenbesuchenden Ameisen sind auch beliebige Rhythmen dressierbar. Hier fällt das regelmässig sich wiederholende Zeitintervall nicht mehr zusammen mit einer täglich wiederkehrenden Tagesstunde. Grabensberger sieht in der Fähigkeit, beliebige rhythmische Anstösse in der Zeit beizubehalten, eine bei den Insekten weitverbreitete Eigenschaft, die bei den ausschliesslich auf Blüten-besuch angewiesenen Bienen zu einem starren System im 24-stündigen Rhythmus geworden ist.

Im Gegensatz zu Experimenten, die sich mit Dressuren auf ganz bestimmte, täglich wiederkehrende Tagesstunde oder auf gleichmässig sich wiederholende Zeitintervalle beschäftigen, stehen Versuche, die von der Frage ausgehen, ob Tiere kurze Zeitstrecken *ohne* den Rahmen der täglichen Wiederholung nach ihrer Dauer abgrenzen und miteinander vergleichen können. Wie weit Zeitgedächtnis und "Zeitschätzungsvermögen" ineinander übergehen, wird noch das Ziel weiterer Forschung sein müssen.

(1) *Untersuchungen mit weissen Ratten.*

Über die Fähigkeit, den Ablauf kurzer Zeitstrecken zu schätzen, die nicht in rhythmischer Folge geboten werden, liegen bei weissen Ratten interessante Ergebnisse vor. Crawford und Tolman (1925) hielten weisse Mäuse in Käfigen, die je zwei gleichlange Gänge bis zur Futterkammer hatten. Die Ratten mussten zwischen dem rechten und dem linken Gang wählen, um zum Futter zu gelangen. Wählten sie den rechten Gang, so wurden sie durch zwei Falltüren beim Durchlaufen eine Minute gefangengehalten, ehe sie in den Futterraum durften. Wählten sie den linken Gang, so mussten sie zwischen den Falltüren sechs Minuten warten, ehe sie weiterlaufen konnten. Die Ratten begriffen sehr schnell, wo sie die kürzere Zeitstrecke abwarten mussten, und bevorzugten deutlich diesen Durchlauf. Wurden die "Wartezeiten" gegeneinander ausgetauscht, so gingen die Ratten schon beim nächsten Mal den anderen Gang. Sie können also sehr wohl Zeitstrecken voneinander unterscheiden. Interessant ist die Feststellung, dass nicht alle Versuchstiere dieselbe Exaktheit in der Zeitschätzung besassen. Dass weisse Ratten Zeitstrecken abschätzen lernen können, bestätigen auch die Versuche von Anderson (1932). Anderson wählte dieselbe Versuchsanordnung, wie sie soeben beschrieben wurde, nur hatten die Ratten hier zwischen vier Gängen zu wählen. Je nach der Wahl mussten sie vor dem Eintritt in die Futterkammer 1, 2, 3, oder 4 Minuten vor einer vorgeschobenen Türe warten. Die Tiere lernten die verschiedenen Zeitstrecken voneinander zu unterscheiden, wobei das Weber'sche Gesetz annähernde Gültigkeit zeigte. Wenn die 4 Zeitabschnitte stufenweise angeordnet wurden, so erlernten die Ratten sie besser zu unterscheiden, als wenn sie in unregelmässiger Folge geboten wurden.

(2) *Untersuchungen mit weissen Mäusen.*

Die Versuche, die Beling (1929) unternahm, um festzustellen, ob weisse Mäuse imstande sind, den Ablauf einer Stunde zu schätzen, verliefen negativ. Beling brachte einzelne Mäuse in einen Käfig, dessen gleichgrosse Hälften mittels eines Durchlaufes in Verbindung standen. Ein automatisches Schaltwerk setzte alle Stunden den Boden eines der beiden Räume unter schwachen elektrischen Strom. Durch diesen unangenehmen Reiz vertrieben, musste die im Käfig befindliche Maus alle Stunden ihren Aufenthalt von einem Raum in den andern verlegen, von dem sie nach Ablauf einer Stunde wieder flüchten musste, und so fort. Eine Übermüdung des Versuchstieres war bei dieser "Dressur" nicht zu befürchten, da Mäuse nach den Untersuchungen Scymanski's (1914, 1916, 1920, 1922) polyphasisch sind, d. h. *viele* Ruhe- und Aktivitätsperioden im Laufe des 24-stündigen Tages aufweisen. Nach sechs Wochen lang anhaltender "Dressur" zeigten die Mäuse noch immer keine Unruhe nach Ablauf einer Stunde in Erwartung des unangenehmen Reizes. Erst wenn dieser einsetzte, flüchteten sie in den anderen Raum hinüber, obwohl es durchaus möglich gewesen wäre, sich vor Eintritt des Stromwechsels auf der Schwelle der Durchgangspforte vorher in Sicherheit zu bringen. Das Verhalten wich nicht ab von dem Verhalten einer nicht "vorbehandelten" Maus,

die 24 Stunden lang in den stromlosen Käfig gesetzt worden war, und als Kontrolle
auf ihr Verhalten im Käfig hin beobachtet wurde. Nach diesen Untersuchungen
können weisse Mäuse anscheinend nicht auf einen regelmässigen Rhythmus von
24 Stunden dressiert werden, und sind nicht imstande, den Ablauf einer Stunde zu
schätzen.

(3) *Das Schätzen kurzer Zeitspannen beim Menschen.*

Mit Zeitschätzungsversuchen am Menschen befasste sich in jüngster Zeit
v. Skramlik (1934). Zunächst stellt er allgemein fest, dass dem Menschen ein eigener
"Zeitsinn" fehlt. Das vom Menschen gewählte Zeitnormal zur Beurteilung des
Zeitablaufes wurde den astronomischen Verhältnissen entlehnt, nämlich dem 24-
stündigen Sterntag. Die astronomische Zeiteinheit ist die Sekunde. Dieses Hilfs-
mittel ist notwendig geworden, da wir an unseren organischen Lebenserscheinungen
keine Anhaltspunkte haben. Sie sind zwar rhythmisch, doch nicht im astrono-
mischen Sinne, denn bei ihnen wiederholt sich das gleiche Geschehen nicht in
durchaus gleichen Zeiten. Dies gilt z. B. für die Herzfrequenz, die Atemfrequenz,
die Periodendauer der Pendelbewegungen des Zwölffingerdarmes. Nach v. Skram-
lik besitzt der Mensch für die Schätzung abgelaufener Zeit nur die Erfahrung durch
Einüben; das Gedächtnis spielt danach bei der Einprägung eines astronomisch
festgelegten Zeitwertes eine Rolle. Wie er feststellte, hängt Genauigkeit der
Einprägung von äusseren und inneren Umständen, vor allem von der Gemütsver-
fassung ab. Frohe Stunden erscheinen uns bekanntlich kürzer als die Stunden der
Depression. Von Skramlik stellte Zeitschätzungsversuche an, die sich auf Minuten-
werte, Sekundenwerte und Bruchteile von Sekunden erstreckten. Die meisten
Versuchspersonen gerieten zunächst in einen Zustand der Verwirrung und der
Hilflosigkeit, wenn sie kurz nach dem Versuchsbeginn Angaben über die verstri-
chene Zeit machen sollten. Die Zeitstrecken wurden von den Versuchspersonen
teils über- teils unterschätzt. Eine dritte Gruppe von Versuchspersonen schwankte
zwischen gleichzeitigem Über- und Unterschätzen der verflossenen Zeit. Es zeigte
sich bei den eingehenden Versuchen, dass die *Minute* am besten geschätzt werden
kann. Die Versuchspersonen stellten sich bei der Schätzung als Hilfsmittel die
Pendelbewegungen einer Sekundenuhr vor, oder sie behalfen sich durch rhythmische
Handbewegungen, Atembewegungen, oder durch Zählen. Durch Üben konnten
die Ergebnisse stets deutlich verbessert werden, doch blieben die Erfolge in der
Einprägung der Zeiteinheit trotz dieser Hilfsmittel nicht lange bestehen. Die
Zeitschätzung wird umso ungenauer, je kürzer die Zeitstrecke ist, die abgegrenzt
werden soll. Bruchteile von Sekunden wurden ebenso ungenau geschätzt wie die
Sekunde selbst. Das Abgrenzen von einer Sekunde (der astronomischen Zeiteinheit)
stiess ausnahmslos auf Schwierigkeiten.

Von Skramlik schliesst daraus, dass dieser Zeitwert für den Menschen nicht
adäquat ist. Dass Zeiträume, die länger als eine Sekunde sind, ganz allgemein
besser abgegrenzt werden können, beruht nach seiner Ansicht darauf, dass das
Zählen im Sekundenrhythmus zum Bestimmen einer Minute niemals genau vor
sich geht. "Die verschiedenen Abweichungen nach oben und unten heben sich

zuletzt zugunsten eines annähernd richtigen Minutenwertes auf" (S. 101 ff.). Im Gegensatz zu dem *motorischen* Einprägen einer Zeitstrecke stehen die Versuche v. Skramlik's zum *sensorischen* Einprägen von Minuten, Sekunden, und deren Bruchteilen. Auch bei diesen Versuchen, wobei es sich um das Schätzen einer Zeitstrecke mit Gehör, Gesicht und Getast handelte, konnte durch Lernen das Ergebnis verbessert werden. Die Sekundenwerte wurden bei den "sensorischen" Versuchen besser abgegrenzt, wie bei den "motorischen" Versuchen. Die Abweichungen von der richtigen Schätzung einer Sekunde betrugen beim motorischen Schätzen ± 5 %, beim sensorischen Schätzen nur ± 1 %. Hinsichtlich des Geschlechtes der Versuchspersonen fanden sich beim sensorischen Einprägen von Zeitwerten folgende Unterschiede: Frauen lernen insbesondere das akustische Abgrenzen, Männer das optische objektiv richtig.

Auf Grund der vorliegenden Untersuchungen vergleicht v. Skramlik unsere "der objektiven Zeiteinheit angepasste subjektive Zeitauffassung" mit dem Gang einer Uhr und nennt sie daher "die physiologische Uhr." Diese physiologische Uhr ist ausserordentlich schlecht, denn sie geht einmal vor und einmal nach. Dazu kommt ihre grosse Labilität durch nervöse Umstimmungen, sodass v. Skramlik zu dem folgenden Schlusse kommt: "Wegen dieser leichten Beeinflussbarkeit erweist sich also unsere innere Uhr zu jeglichen genauen Messungen als völlig ungeeignet" (S. 105).

V. SCHLUSS.

Nach den vorliegenden Untersuchungen kommen wir zu dem Ergebnis, dass das "Zeitgedächtnis" oder, wie vielfach auch gesagt wird, der "Zeitsinn" in seinen Erscheinungsformen vielfach verwickelte Aufgaben an unser Verständnis stellt. Vor allem kommen wir in Verlegenheit, wie wir zusammenfassend das Problem benennen sollen. Denn wir kennen kein Sinnesorgan für die Perzeption der Zeit, sodass der Name "Zeitsinn" uns unberechtigt erscheint. Und weiterhin ist es—im Falle der sozialen Insekten—unberechtigt, von einem "Zeitgedächtnis" zu sprechen, da der Sitz dieses "Gedächtnisses" *nicht*, wie der Name andeutet, im *Gehirn* zu suchen ist. Beim Zeitschätzungsvermögen des Menschen und der daraufhin untersuchten Tiere (Mäuse) dagegen haben wir es deutlich mit Gedächtnisfunktionen zu tun.

VI. ZUSAMMENFASSUNG.

1. Viele Organismen folgen in ihren Lebensgewohnheiten kosmischen Periodizitäten. So haben sich z. B. manche Meeresorganismen dem regelmässigen Wechsel der Gezeiten angepasst, indem sie während bestimmter Stunden Schutzreaktionen ausführen. Viele Vögel folgen in ihrem Verhalten dem Wechsel der Jahreszeiten. Das bekannteste Beispiel für den Einfluss kosmischer Rhythmen ist die unter den Lebewesen weit verbreitete Anlehnung an den periodischen Lichtwechsel; es ist dies der dem Wechsel von Tag und Nacht angepasste Wechsel von Ruhe und Aktivität.

Diese "Biologischen Rhythmen" treten vielfach auch dann noch einige Zeit selbständig in Erscheinung, wenn durch konstante Bedingungen in einem

Versuchsraum die direkten Einflüsse der kosmischen Periodizitäten ausgeschaltet werden. Ihr Ablauf fällt in vielen Fällen—trotz Wegfalles des gewohnten periodischen Reizes—noch "pünktlich" mit dem zeitlichen Ablauf der Aussenfaktoren zusammen. Dieses Zeitgedächtnis ist nicht variabel, sondern streng gebunden an den "eingelernten" Rhythmus. Ob es seine Entstehung einer erblich oder individuell erworbenen "Erinnerung" verdankt, ist noch unbekannt.

2. Eine höhere Stufe stellt das Zeitgedächtnis einiger sozialer Insekten (Bienen, Ameisen, Termiten) dar. Hier ist der Organismus nach voraus gegangener "Dressur" imstande, im Rahmen des 24-stündigen Tages jede beliebige Stunde zu bestimmen. Wir haben hier also ein variables Zeitgedächtnis, das, wie nachgewiesen, von äusseren Periodizitäten ganz unabhängig ist. Das Zeitgedächtnis der genannten Insekten ist zahlreichen Untersuchungen unterzogen worden, welche das ganze Problem entscheidend gefördert haben. So wissen wir heute, dass die "Uhr," die dieses verblüffend präzise Zeitgedächtnis bestimmt und entscheidend beeinflusst, die Geschwindigkeit des Stoffumsatzes im Körper ist. Es wird angenommen, dass diesem ausgesprochenen Zeitgedächtnis eine biologische Bedeutung im Zusammenhang mit dem Nahrungserwerb dieser sozialen Insekten zugrunde liegt. Eine entsprechende Fähigkeit ist uns bisher bei keiner anderen Tiergruppe in diesem Ausmasse bekannt.

3. Die dritte Stufe des Zeitgedächtnisses ist die Fähigkeit, bestimmte Zeitstrecken frei nach ihrem Ablauf zu schätzen, wenn sie nicht im Rahmen einer regelmässigen Wiederholung und Reihenfolge als "Dressur" geboten werden. Derartige Versuche wurden bisher nur mit der weissen Ratte und dem Menschen angestellt. Bei beiden konnte die Fähigkeit zum Abschätzen kurzer Zeitspannen bis zu einem gewissen Grade nachgewiesen werden.

VII. SUMMARY.

1. The habits of many living organisms are subject to cosmic periodicities. A number of marine animals, for example, are adapted to the ebb and flow of the tides, and many birds follow the changes of the seasons in their behaviour. The most familiar example of the influence of cosmic rhythms is the widespread dependence of living organisms on periodic light changes; their alternations of rest and activity correspond with night and day.

These biological rhythms frequently continue after the direct influence of cosmic periodicities has been experimentally removed. In spite of the absence of the usual periodic stimulus, the course of a biological rhythm may correspond punctually with the time sequence of the external factors. It is unknown whether such rhythms are due to inherited "memory" or are individually acquired.

2. The time memory of certain social insects (bees, ants, termites) is on a higher level. These animals, after "training," are capable of determining any hour of the day and their time memory is independent of external periodicities. The "clock" which here determines the hour with such remarkable precision has been shown to be the rate of metabolism in the body. It is assumed that this time memory has a meaning in connection with the acquisition of food by these insects. No corresponding time memory is known in any other group of animals.

3. The third level of time memory is the capacity of estimating periods of time, without the latter having been impressed on the animal by "training." Experiments to test this faculty have up to the present been performed only with white rats and with man. In both cases the ability to estimate short time intervals has been proved to exist, up to a point.

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MITOGENETIC RADIATION

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CONTENTS.

I. INTRODUCTION.

THE literature on mitogenetic radiation has expanded to enormous proportions in the twelve years since the publication of Gurwitsch's original papers in *Arch. EntwMech. Org.* Many writers have asserted dogmatically that the existence of the radiation has long been beyond all possible doubt, and the members of Gurwitsch's school, acting on this assumption, have continued to produce discoveries at an alarming rate, without pausing to reconsider the validity of their fundamental experiments and sometimes without paying very much attention to what they wrote some years ago. Nevertheless, much criticism, experimental and theoretical, has appeared, and few onlookers would share the optimism of the Russian workers with regard to their fundamentals. Equally few would venture to say that anyone has finally disproved the existence of these troublesome rays, or could do so. Because of this final uncertainty it seemed

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that one could as well make a working decision on the matter now as at any other time, and this survey has been written with the object of evaluating the available evidence. I have confined my attention to work which furthers this object and have made no attempt to produce a complete catalogue of the phenomena described in mitogenetic literature, nor to give an exhaustive bibliography.

II. THE ORIGINAL MITOGENETIC EFFECT.

(1) *The fundamental arguments.*

In 1922 Alexander Gurwitsch was led to seek for a new mitosis-stimulating factor different from the "Teilungshormone" of Haberlandt. He had previously (see 1922) concluded that two factors are involved in the stimulation of cell division: an internal predisposition to divide ("Möglichkeitsfaktor"), and an external stimulating factor ("Verwirklichungsfaktor"). As evidence for the existence of the latter, he showed that root tips of *Helianthus* when physiologically separated from the sole, but uninjured, did not continue to grow. His further arguments are based on the observation that in the same tissue division of a cell becomes linearly less probable as the cross-section increases, and he concludes that it is the cross-section which determines whether or not the Verwirklichungsfaktor shall be effective. How can the cross-sectional area be of such decisive importance? Arguing quasi-mathematically, Gurwitsch shows that it can be regarded as having two components: the original cross-section C, which is constant, and the increment A which represents the cell's growth and is an exponential function of its age. If the C component is reactive to the Verwirklichungsfaktor and the A component is not, then the greater the proportion of A, the less favourable are the conditions for cell division. Now if a uniform hormone concentration were present, and if only the value of C/A determined the cell's reaction to it, then this reaction should not be subject to statistical variation. On this ground Gurwitsch rejects the hormone theory. He then explains the statistical nature of the reaction by allowing the C and A portions of the surface to preserve their geometrical identity, postulating that different geometrical configurations of C and A patches will determine differing reactions to the same stimulus. A resonance phenomenon suggests itself: "Sobald wir in unsere Betrachtung die Annahme einführen, dass der Faktor der Reizperzeption ein bestimmt konfiguriertes Etwas von bestimmter Grösse in der Zelloberfläche ist, drängt sich von selbst eine weitere Vermutung auf: *Erfolgt nicht etwa die Reizperzeption als ein der Resonanz wesensgleicher Vorgang?*" (Gurwitsch, 1923). And a resonance phenomenon implies an oscillatory stimulus.

(2) *The fundamental experiments.*

The first experimental tests were made with injured frog's cornea. A small round burn caused within four days a powerful mitotic reaction over a region 4–5 mm. in diameter, which might have been due to the diffusion of a Wundhormon. But if a very slight linear wound was made with a warm platinum wire at the same time as the round one, the former screened the mitosis-stimulating factor and the

resulting "shadow picture" was said to demonstrate its linear propagation (Gurwitsch, 1923). A more elegant confirmation was obtained in experiments with onion roots, on the supposition that the stimulating radiation originates in the sole and then passes axially along the root. In a straight root, accordingly, the distribution of mitoses in the meristem was stated to be symmetrical, to within 1·5 per cent., about the longitudinal axis. If now the root were bent, the rays should be totally reflected at the bend, and the distribution of mitoses should become unsymmetrical in a way which could be predicted geometrically. Geometrical expectations were invariably fulfilled. "Von ganz besonderem Wert ist es dabei, dass es in der Hand des Experimenters liegt, den Versuch so anzuordnen, dass das Übergewicht bald an der konkaven, bald an der konvexen Seite zu gewärtigen wäre, wobei die Vorhersage sich ausnahmslos bestätigt" (Gurwitsch, 1924, p. 59). It follows that a bundle of rays should emerge from the tip of a straight root, the unabsorbed residue of the bundle emitted by the sole, some centimetres distant. An attempt was made to detect this residual radiation with a second onion root, assuming that a superimposed stimulus would cause an increase in the number of mitoses in the second root[1].

A small area of the meristem of one onion root was exposed to the tip of another, and mitoses on each side of longitudinal sections of the induced root were counted. The result was that "es besteht ein sehr bedeutendes systematisches, scharf circumscriptes, auf das zentrale Gebiet der Wurzelspitze beschränktes Übergewicht an der induzierten Seite": namely, in seven or eight median sections (Gurwitsch, 1924, p. 64). This was the famous "Grundversuch."

The importance of this experiment warrants a more detailed description (A. and L. Gurwitsch, 1925 a, Gurwitsch, 1929 b). The detector root must be 5–6 cm. long, the distal end straight, and the tip symmetrical. Attached to the lower part of the onion, it is passed carefully through a tapering glass tube, and the tip held by a second tube 2–3 mm. below the first. 150 blank experiments showed that this procedure has no effect on symmetry of mitoses, provided the root does not rub against the glass. The inducing root must be about 4 cm. long and quite straight, in order that the rays may not be made diffuse by internal reflection. It is passed through a horizontal tube and microscopically "centred" with respect to the detector. The distance between the two roots is 1·5–2 mm. After induction the direction of the radiation is marked by passing a fine needle through the detector, which is then laid on a flat board with the needle horizontal and about 6 mm. of the root tip cut off obliquely. The tip is fixed at once in Bouin, embedded in paraffin, and cut into 10μ longitudinal sections parallel to the direction of induction. These are stained with iron haematoxylin. This procedure has the advantage that the resting nuclei are completely decolorised by the differentiation in iron alum, while the chromatin threads in the very earliest mitotic phase are sharply stained. The mitoses, from the earliest prophase to the telophase, are counted in the two halves of each section, the dividing line being fixed by inspection under low power.

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[1] Obviously a more satisfactory test would be to find whether the proximity of a root tip could cause resumption of mitotic division in a root in which this had been stopped by inhibition of the normal mitogenetic stimulus (see Gurwitsch, 1922; Wassermann, 1931).

Although the excess of mitoses on the induced side occurs almost entirely in the dermatogen and periblem, Gurwitsch also counts the plerom, since it is not easily distinguished from periblem. For a similar reason the calyptrogen is included.

The counts are made several times by two observers, whose results generally agree to 1–2 per cent. Duplicate counts of three single sections are quoted, and a very unsatisfactory control experiment (an unsuccessful induction experiment!) is given by Rawin (1924). In Gurwitsch's original paper five protocols of successful experiments, distressingly lacking in detail, are published, showing the positive effect (~30 per cent.) in seven to eleven successive median sections, and the number of mitoses in the remaining sections; in two experiments, where source and detector were in contact, the positive zone was more extensive. Numbers of less detailed protocols appear in later papers.

It appears that each section was counted along its entire length. This makes the observed positive effects (sometimes as great as 50 per cent.) astonishing, since a large part of every section—plerom in the induced region and all cells in the uninduced region— could not contribute to the induction effect.

One would also have expected that as the rays can be transmitted axially along a root without significant decrement, the result of induction would be an increased number of mitoses in the entire cross-section in the irradiated zone. Since the effect only appears on the induced side, and principally in the dermatogen, the onion root must be quite remarkably anisotropic—a fact which has not escaped Gurwitsch's attention, but which apparently causes him little surprise.

(3) *Confirmations and criticisms.*

Those who have been concerned to prove or disprove the existence of mitogenetic radiation have given most of their attention to the Grundversuch, ignoring the theory which led to it and the earlier experiments. But the first comment to appear was that of Frederikse (1927–8), who dealt with these topics. He remarked that in the cornea experiments the function of the linear wound was obscure, that Gurwitsch's shadow pictures were devoid of shadows[1], and asked why the onion root showed no Tyndall effect. He repeated the cornea experiments in such a way as to exclude hormonal agencies. The wound was made in the nictitating membrane of a frog, sewn down over the eye, the other eye remaining as control, and after 6–10 days the corneal mitoses were counted. The result of ninety-three such experiments was that the wound had no effect (cp. Gurwitsch, 1929a).

The Grundversuch itself has often been repeated, but only Moissejewa (1931a,b) has adhered to the Gurwitsch technique. An idea of the wide divergence in technique and in results can be obtained from Table I.

Before considering the criticisms which have been made, it is desirable to state clearly the position with regard to the Grundversuch. Gurwitsch asserted the existence of a certain phenomenon. He published insufficient details of his evidence, but he insisted that the phenomenon is a reproducible one, hundreds of experiments

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[1] This statement can be confirmed by reference to Gurwitsch (1924, p. 55).

made in his laboratory having given the same result. "Sowohl die positive Ergebnisse, als die Nulleffekte traten in jeder Kategorie unserer Versuche in der Regel in 100 Prozent der Fälle auf" (Gurwitsch, 1928). Now if a number of competent workers repeat his experiment *as exactly as possible* and fail to obtain the same result, it may be assumed that the phenomenon is imaginary: scientifically, moreover, the reason for the original assertions is uninteresting. A second possibility is that the observations, though reproducible, may be demonstrably dependent upon accidental asymmetry, and a third is that they are correct.

Examining the criticisms of Gurwitsch's work from this angle, it must be admitted that none can be considered legitimate. If, for example, the phenomenon is a constant one in Gurwitsch's hands, then his technique is adequate and mere technical criticisms have only technical interest. The remarks of v. Guttenberg and Rossmann (Guttenberg, 1928 *a, b*; Rossmann, 1928) are of this kind: they assert, perhaps with justice (although it is disputed by Wassermann, 1931; Paul, 1933; and Gurwitsch, 1928), that nuclei in one of the phases included in Gurwitsch's counts— the early prophase—are difficult to distinguish from resting nuclei, so that the inclusion of this phase is a source of error. Again, v. Guttenberg (1928 *b*) points out that the number of sections counted in Gurwitsch's experiments is often insufficient: in sixty experiments out of 110 it was less than ten. But without the addition of some personal factor, such errors could not account for consistent positive results.

Rossmann's experimental work gives good evidence against an induction effect, but suffers from the defect that the technique differs from Gurwitsch's (see Table I). Gurwitsch naturally retorted (1928) that these technical divergences were responsible for the negative results: that the induction effect shows itself principally in the early prophase, not counted by Rossmann, because the time of induction used is not very different from the interval between earliest prophase and early metaphase, and that 40 per cent. of the total excess of mitoses is situated in the dermatogen, not counted by Rossmann. v. Guttenberg pointed out the incorrectness of this last statement on account of the very small number of cells in the dermatogen, and Gurwitsch has since modified his statement to apply to "dermatogen and periblem" (1929 *b*). His reply to the first statement remains unassailed: Rossmann's negative evidence is not decisive.

The remaining important critical work is that of Moissejewa (1931 *a, b*, 1932), who learnt the technique in Gurwitsch's laboratory. She then set up an identical apparatus in Kiew, which was inspected and approved by one of Gurwitsch's pupils. Her experiments were very convincingly negative. On the purely critical side, her work, like Rossmann's, does not get very far. She showed that the slightest pressure or stroking on the meristematic zone of an onion root causes asymmetry in the distribution of mitoses, and that slight bends in the proximal part of the root can have a similar influence; irregularities could only be avoided by handling the roots with the greatest care. Obviously such disturbances occurring accidentally in Gurwitsch's experiments *could* upset the results: Moissejewa suggested that the performance of the experiments according to an empirical plan ensured the production of a constant asymmetry in the expected direction. Frank and Salkind

(1932) replied categorically "Keine der Annahmen der Autorin über die Methodik von Gurwitsch zutrifft." Gurwitsch (1931c) added: "—es wäre absolut unerklärlich, warum in den in der Literatur geschilderten über 200 positiven Versuchen die unbewusste Reibung gerade am Induktionsort geschah und in den etwa 130 Versuche mit Nulleffekte die Symmetrie bewahrt blieb und folglich jede mechanische Beeinflussung fehlte." But he refused to discuss Moissejewa's excellent experiments.

The various confirmations of the Grundversuch have only their optimistic conclusion in common; the positive effect manifests itself in discouragingly diverse ways. For example: (1) Magrou and Magrou (1927), Magrou (1931) found the excess of mitoses to be localised *inside* the first six layers of cells, *i.e.* in the inner part of the periblem; Gurwitsch and Loos find it in the peripheral zone; Paul finds it to be about equally distributed between (dermatogen – calyptrogen + outer periblem) and (inner periblem – plerom). (2) The induction effects found by Gurwitsch and by Reiter and Gabor are often produced by a *decrease* in mitoses on the uninduced side (see Rossmann, 1928, p. 365; Reiter and Gabor, 1928, p. 13), a fact which Reiter and Gabor attempt to explain theoretically, while Gurwitsch (1931c, p. 198) now says that this occurs in *not more than half* of his own experiments and that in such cases, for technical reasons, plerom was excluded from the counts of the median sections and included in the others. (3) Gurwitsch finds the induction effect to be sharply localised laterally—in a region about 70μ wide, about the width of the supposed parallel bundle of rays—but to extend longitudinally over the entire meristem; in Wagner's results[1] it is more diffuse laterally (possibly because of movement of the roots during induction); Paul finds it to extend laterally over the whole root (1933, Tab. 4) but to be limited to about $\frac{1}{3}$ mm. longitudinally; Reiter and Gabor find it to be limited to about 150μ longitudinally.

The recent work of Paul (1933) demands special consideration, because the author has developed a technique which promises to provide a final decision on this question. Longitudinal sections through the meristem are individually copied with a projection apparatus, the positions of the mitoses (eleven distinct phases being recorded, following Tischler) recorded half-schematically, and the successive sections then superimposed. From the resulting "map" the distribution of mitoses in any type of section can be determined, and hence the configuration of any induction effect. The actual experiments given show the excellence of the method, but although Paul draws positive conclusions from them, I think they can only be regarded as preliminaries. Only two roots were induced; the inducing roots served as controls. Both source and detector were kept in exactly the positions in which they had grown (cp. Wagner, 1927, 1928), still attached to onions with the remaining roots intact. The upper sides of the roots were induced. The results of both experiments showed a marked excess of mitoses on the induced side; this exhibited itself in all longitudinal sections, almost equally in the peripheral and central halves of these sections, and in transverse sections over $200-800\mu$. The two controls showed

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[1] Wagner's data are taken by Rossmann as providing evidence *against* Gurwitsch, and they are not recognised by Gurwitsch himself.

no such regular difference, and the author concludes that an induction effect was present. This may be disputed, because both control and induced roots had also a considerable asymmetry in a plane at right angles to the plane of induction. The induced roots would therefore have shown "induction" effects, whatever the direction of the longitudinal sections. The effect observed has no necessary connection with the presence of the inducing root. The presence of a similar side-to-side asymmetry in the control roots is taken by Paul as sufficient reason for ignoring it in the induced ones. Since the remaining experiments, in which roots were induced with knitting needles, showed no induction effects, they may be taken as additional controls. These roots show far larger variations than those just discussed, for this reason supporting one's belief that more extensive data are necessary before an induction effect can be established.

Paul obtained some indication of a macroscopic induction effect: tendency of roots to move away from other approaching roots, or from knitting needles (cp. Petri, 1928; Elfving, 1890; Byrükov, 1926; Rossmann, 1928).

It must be concluded that a final decision concerning the Grundversuch cannot yet be made.

III. PHYSICAL CONSIDERATIONS.

(1) *Some physical properties of mitogenetic radiation.*

Mitogenetic radiation, according to the early experiments, originates in the sole, passes with little loss of intensity[1] along the vascular bundle, and emerges from the root tip as a parallel beam about 70μ wide—judging at least from the lateral extent of the region which it influences in the detector root. (Judging from the longitudinal extent of this zone of action we would have to suppose the shape of the beam to be a function of the observer). The origin of the rays in the sole has been demonstrated by L. Gurwitsch (1924), who found that the narcotised root, completely mitosis-free, still radiates, while a normal root attached to a narcotised sole does not. The nature of the axial beam is easily investigated by cutting off narcotised roots[2] at various levels: a short root stump emits a narrower beam than a long one. The axial beam, in other words, is slightly divergent. Arriving at the conical tip, the peripheral rays are internally reflected into the periblem and dermatogen, where they stimulate mitoses; the central rays, incident normally on the bounding membrane, are able to escape into the outer world (Gurwitsch, 1924). In agreement with this, no radiation could be detected from a bent root, while root tips 1·5 mm. long were transparent axially to mitogenetic radiation (Gurwitsch, 1926, p. 74; according to Reiter and Gabor (1928, p. 104), a dead root is not entirely opaque transversely to the rays, although a living one, according to Gurwitsch, is).

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[1] Some loss of intensity is inferred from the fact that short detector roots respond less readily to external mitogenetic stimulation than long ones (A. and N. Gurwitsch, 1924).

[2] If the root is not narcotised, the sole suffers from surgical shock and refuses to radiate. But all roots of an onion except one may be amputated without affecting the radiating capacity of this one. Which shows us that each root has its own independent physiological centre in the sole! (Rawin, 1924; L. Gurwitsch, 1924).

These remarkable properties do not help much towards identifying the mito-genetic rays with any known type of radiation. Fortunately other experiments gave a more familiar picture: the rays were reflected by glass (Gurwitsch, 1924), trans-mitted by 25μ glass (Rawin, 1924), but largely absorbed by 0·1 mm., transmitted completely by 3 mm. crystal quartz (A. and L. Gurwitsch, 1925c). From this evidence, ignoring the transparency of onion roots, Gurwitsch inferred that mito-genetic radiation is identical with ultra-violet light of wave-length 190–230$\mu\mu$ (see also Siebert, 1931; Wolff and Ras, 1931, 1932a).

This conclusion was not supported by the experiments of Reiter and Gabor (1928). They agreed about the reflection of the rays, but found them to be trans-mitted by quartz and by 3 mm. glass, 0·2 mm. gelatin, and 1 mm. uviol glass, concluding that the wave-length must be greater than 300$\mu\mu$. They confirmed this by absorption experiments with a p-nitrosodimethylaniline and fuchsin filter and finally by a spectral analysis of the radiation; an onion root placed in the spectrum gave a positive effect at 340$\mu\mu$.

Referring to the wave-length discrepancy, Gurwitsch (1929a, p. 469) says with justice "dass wir m.E. vorläufig vor einem ungelösten Rätsel stehen."

(2) *The detection of mitogenetic rays by physical and physico-chemical methods.*

If, with Gurwitsch, we accept certain experimental results and choose to ignore certain others, it is possible to conclude that mitogenetic rays are identical with short ultra-violet. In this section the physical evidence for this conclusion will be discussed.

Photographic and photo-electric methods. The photographic method is the simplest one for detecting radiation. It possesses the advantages of high sensitivity and ability to summate light stimuli, but it can also summate weak chemical stimuli, which are extraordinarily difficult to exclude (see, *e.g.*, Russell, 1898; Mathews and Dewey, 1913; Taylor and Newton Harvey, 1931). There is a strong suspicion that such disturbances were present in some of the reported positive experiments, and in any case they (Protti, 1932; Brunetti and Maxia, 1930; Maxia, 1933b; Vaccari, 1932; Čech, 1932a,b; Reiter and Gabor, 1928; Lepeschkin, 1933) are offset by just as many negative results, which in general carry more conviction (Taylor and Harvey, 1931, 89 days' exposure; Rossmann, 1928; Loos, 1930; Petri, 1928; Elger, 1932; Magrou, 1930; Hauer, 1932).

The only other physical methods which have the necessary sensitivity are based on the photo-electric effect. Use of an ordinary photocell has been reported, without details, by Chariton, Frank and Kannegiesser (1930) with negative results. Schreiber and Friedrich (1930) followed with an unsuccessful attempt to detect the radiation from yeast with an argon-filled potassium cell. This was replaced by a more sensitive arrangement, a variant of the Elster and Geitel method (1916), which also gave negative results, although it should have detected the radiation emitted even if each dividing yeast cell only gave one quantum of ultra-violet radiation (wave-length 266$\mu\mu$) every 200 sec. (see Table II). A paper by Potozky (1930), appearing shortly

Table II. *Detection of mitogenetic radiation by photo-electric methods.*

Author	Method	Photo-sensitive material	Photo-electric yield (quanta per electron emitted)	Calibration wave-length $(\mu\mu)$	Conclusion $(+ \text{ or } -)$	Estimated intensity of mitogenetic radiation $(h\nu/\text{cm.}^2\text{sec.})$
Chariton, Frank and Kannegiesser (1930)	Photocell	K	?	—	–	$< \sim 10^5$
Schreiber and Friedrich (1930)	Photocell used as Geiger counter	K	$2 . 10^4$	254	–	$< \sim 10^3$
Rajewsky (1931)	Geiger counter	Cd	$\sim 10^2$	265	+	14–140
Frank and Rodionow (1932)	,,	Cd, Al	$2 . 10^3$	254	+	600–2000
Seyfert (1932)	,,	Zn amalgam, Al, Mg	?	—	–	?
Locher (1932)	,,	Various	?	—	–	?
Vaccari (1932)	Photocell	K	?	—	–	?
Petri (1932)	,,	Cd	?	—	+	?
Gray and Ouellet (1933)	Geiger counter	Pt	$6 . 10^3$	254	–	< 50
Lorenz (1933, 1934)	,,	Cd	$4 . 10^2$	254	–	< 10
Siebert and Seffert (1933)	,,	?	—	—	+	?
Kreuchen and Bateman (1934)	,,	Cd, Zn, Al	$2–5 . 10^4$	254, etc.	–	< 300

after, demonstrated that yeast does not emit mitogenetic rays in the dark. Gurwitsch (1931 a) ascribed Schreiber and Friedrich's failure to this unfortunate circumstance.

Petri (1932, 1933) claims that the rate of discharge of a micro-electrometer connected with a cadmium photocell is increased in presence of germinating wheat. The scanty information given concerning the reproducibility of the dark discharge rate, the effect of moisture, etc., and the absence of protocols compel scepticism.
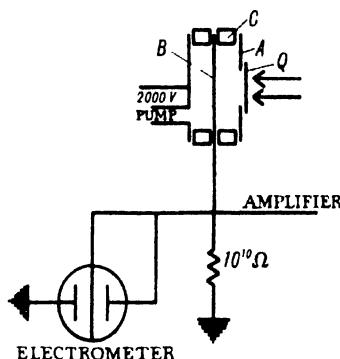
Fig. 1. Simplified circuit of Geiger-Muller counter.

There remain several researches with the photo-sensitive form of the Geiger-Müller counter, first developed by Rajewsky (Dessauer, 1931; Rajewsky, 1931 a, c). The Geiger-Müller tube itself (Geiger and Müller, 1928a; for literature see Kreuchen, 1934) is a device which can record single electrons liberated into the gas space of the apparatus. The metal tube A (Fig. 1) is charged to a high negative potential,

and the axial wire B is earthed through a resistance of about 10^{10} ohms. An electron liberated near the inner surface of the tube is therefore subjected to a strong accelerating field, so that it produces sufficient ionisation by collision during its passage through the gas to permit the occurrence of a discharge between the tube and the wire. The high resistance of the lead to earth introduces a time lag which allows recovery of the insulating properties of the gas within about 10^{-4} sec. The tube is then able to record another electron in the same way. The impulses due to individual electrons can thus be seen as kicks of an electrometer thread connected to the wire, or, after suitable amplification, they can be recorded by some automatic device.

Rajewsky adapted this method for registering photo-electrons, making the tube of a photo-sensitive metal and providing a quartz window. Light of wave-length shorter than the threshold for the metal used caused an increase in the "dark" rate of discharge. Rajewsky's first measurements were made with tubes with ebonite stoppers (C, Fig. 1) and sealed with vacuum-tight cement, so that a sensitive metal surface could hardly have been expected: preparation of the tube involved heating, and probably oxidising, the metal[1]. Nevertheless the electron yield appeared to exceed considerably that of an ordinary photocell (cp. Table II), and it was generally agreed that the method was the best available for the study of mitogenetic radiation. Rajewsky used it for this purpose, obtaining positive results which indicated an intensity between 10^{-9} and 10^{-10} ergs cm.^{2} sec., or, calculated for $265\mu\mu$, about 14–140 $h\nu$/cm.^{2} sec. Glass absorbed the radiation.

Frank and Rodionow (1932) confirmed Rajewsky's result. Their zero effects were very high and variable, doubtless partly on account of the very low gas pressure in the tube—only $1\cdot0$–$1\cdot5$ mm. air or argon. The sensitivity appeared to be about ten times smaller than that of Rajewsky's apparatus, although their positive effects were about the same as those found by Rajewsky. The intensity of mitogenetic radiation implied by their data is about 600–2000 $h\nu$ cm.^{2} sec.

Siebert and Seffert (1933) employed two counters simultaneously, one for exposure to mitogenetic radiation and the other as control, assuming (and claiming, moreover, that their assumption could be justified experimentally) that the statistical variation of the cosmic ray effect is the same in two differently situated tubes. They succeeded in detecting mitogenetic radiation, but their basic assumptions are probably false.

All other published measurements have been negative. Seyfert (1932) used tubes of unsatisfactory design and gave no satisfactory sensitivity data. Gray and Ouellet (1933) used a platinum tube and exposed it to fertilised sea-urchin eggs, commencing their measurements 20 min. after fertilisation and recording the effects at all stages up to completion of the fourth cell division. They concluded that one egg cannot emit, on the average, more than one quantum every 40 sec. Other supposed sources gave the same negative result. Their counter was sensitive to moisture and to vapours emitted by certain biological materials (see also Lorenz, 1933, 1934). Kreuchen and Bateman (1934), using accurately calibrated CO_2-filled

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[1] But cf. Rajewsky, 1934.

tubes of the type drawn in Fig. 2, were able to show that spurious "positive" effects could be obtained if the counter was improperly shielded from the source (and from electrical disturbances accompanying stimulation, in the case of experiments with muscle), in spite of the considerable distance along the glass surface connecting the high potential lead and the central wire. When proper care was taken to exclude such influences, the results were completely negative.

This last investigation, together with the work of Kreuchen (1934), indicated certain limitations of the Geiger counter method. Earlier authors had tended to neglect the difficulties associated with the accurate calibration of the apparatus and had assumed it to be more sensitive than the simple photo-electric cell. Now the calibrations of Kreuchen, in which elaborate pains were taken to exclude stray light and to measure the calibrating radiation with great accuracy, have shown that in none of his counters was the maximum yield of an ordinary photocell exceeded ($\sim 10^4\ h\nu$
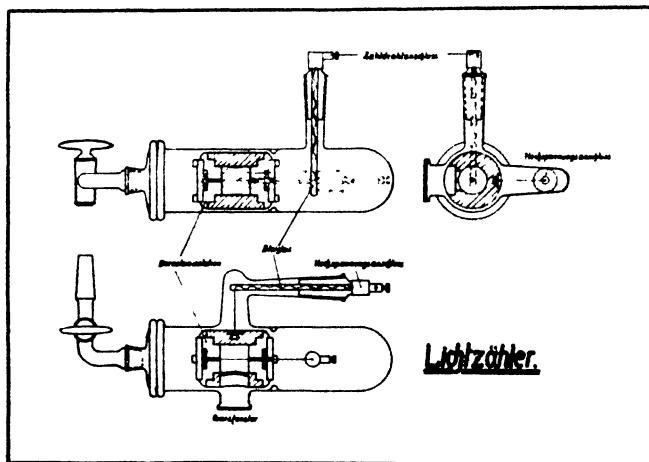
Fig. 2. A photo-sensitive type of Geiger-Müller counter. From Kreuchen and Bateman (1934).

per electron liberated), and there was some indication that as soon as this maximum yield is reached the sensitivity of the counter becomes independent of wave-length. In other words, neither a large quantum nor an applied electrostatic field is capable of increasing the proportion of atoms in the active surface which are at any moment ready to lose an outer electron.

The sole point of superiority of the electron counter lies therefore in its ability to register single elementary processes at the instant of their occurrence. We must draw the disappointing conclusion that the intensity of mitogenetic radiation is below the limit of sensitivity of the most sensitive physical method available—less than about 300 $h\nu$/cm.^{2} sec.*

Other methods. For the sake of completeness, three other physico-chemical methods must be mentioned. The first is the so-called Stempell effect: irregular formation of Liesegang rings under the influence of certain materials which are

* Indoor daylight contains much more short ultra-violet radiation than this (Rajewsky, 1932).

supposed to emit mitogenetic radiation. Stempell (1929) discovered the phenomenon and attributed it to mitogenetic radiation; Tokin (1931, 1933) (Tokin and Baranenkowa, 1931) found that volatile substances were responsible. (See also Czaja, 1930; Gigon and Noverraz, 1930; Maxia, 1930, 1931*b*; Ypsilanti and Paltauf, 1930; Kowarzyk, 1931; Siebert, 1931). Stempell and von Romberg (1931) and Kofman (1932) insist that effects due to radiation can be found when chemical effects are excluded (see the article by Maxia, 1932*a*).

The second method is also due to Stempell (1931*a*, *b*). Germinating peas, it seems, can influence the rate of decomposition of hydrogen peroxide. This effect can hardly be due to radiation. The highest recorded quantum efficiency for the reaction is about 500 (Allmand and Style, 1930)—let us say 1000. And let us assume an intensity of 1000 $h\nu/cm.^2$ sec. for the radiation from peas. We find that in 48 hours (the time required for Stempell's positive effect), with an exposed area of 20 cm.^{2} H_2O_2, about 8 . 10^{-6} cm.^{3} O_2 would be produced. With Stempell's method of measurement, an intensity about ten thousand times greater would be necessary to give a detectable effect.

Finally, Mardaschew and Mogilewsky (1933) find that mitogenetic rays affect the velocity of enzyme reactions.

(3) *The effects of sublethal intensities of ultra-violet light on cell division.*

It was natural that Gurwitsch, believing mitogenetic radiation to be identical with short-wave ultra-violet light, and Reiter and Gabor, believing it identical with long-wave ultra-violet light, should test their respective beliefs by irradiating onion roots artificially. The expectations of both sets of workers were completely fulfilled. Frank and Gurwitsch (1927) used light from an aluminium spark, dispersed in a small quartz spectrograph, finding that 1 min. exposure to lines within the range 193–237$\mu\mu$ produced strong positive effects. Sussmanowitsch (1928), also working in Gurwitsch's laboratory, obtained negative effects ("exhaustion") with longer intermittent exposures. Reiter and Gabor (1928) found positive effects only from 334 to 365$\mu\mu$, with a possible weak maximum at 280$\mu\mu$. Chariton, Frank and Kannegiesser (1930) replied with further experiments on the reaction of yeast cells (in a beer-wort agar medium) to ultra-violet light, confirming the results of Frank and Gurwitsch and failing to find any effect with longer waves. Mixed light, with a wave-length range of 10$\mu\mu$, appeared to be more effective than monochromatic light of the same intensity. In discussing the wave-length discrepancy, Chariton, Frank and Kannegiesser refer to the high intensities used by Reiter and Gabor, and suggest that this would account for their failure to detect an effect with the much more strongly absorbed shorter waves. They quaintly add that since Reiter and Gabor counted only "reife Kerne," they were perhaps not observing a "true" mitogenetic effect. They estimated the threshold amount of radiation required to produce a positive effect with mixed light to be about 16 $h\nu$ per minute per cell, or an intensity 10^5–10^6 $h\nu/cm.^2$ sec. Confirmations of Gurwitsch's view have also been published by Borodin (1931) and Konarsky (1932), both of whom irradiated yeast cells, and by Ruyssen (1933) with bacteria. These results have been disputed by Kreuchen and

Bateman (1934), who exposed suspensions of *Saccharomyces ellipsoideus*, usually in cane-sugar solution, sometimes in KCl or beer wort, to the mercury arc, the intensity being varied accurately over a range $1 : 10^{-8}$ by means of matt quartz plates and a quartz reflector system. The results showed no stimulation of cell division whatever over an intensity range of about 10^5 below the minimum lethal intensity, although the lowest intensity used was roughly $10^9 hv/\text{cm.}^2$ sec. The stated existence of inhibition of growth by excessive doses of mitogenetic radiation excludes the possibility that even $10^9 hv/\text{cm.}^2$ sec. was too great an intensity: at intensities greater than the optimal for the mitogenetic effect we should have observed inhibition.

Many other statements of stimulating effects of ultra-violet radiation of relatively high intensity have been made, often without reference to the mitogenetic radiation problem. Thus (for literature see Beudt, 1930), Bovie (1916) found that the initial effect of Schumann rays on *Amoeba* and Infusoria was an increased motility; Higgins and Sheard (1927) found stimulation of germination and growth rate of the cucumber by wave-length $320-390\mu\mu$; Hinrichs (1928 *a*, *b*) and Grouchi (1932) agreed that ultra-violet light can stimulate the alcoholic fermentation of yeast (but according to Gesenius (1930*a*) mitogenetic radiation *inhibits* it); Suranyi and Vermes (1929) found a 50 per cent. increase in oxygen consumption of avian erythrocytes and yeast cells on irradiation; and various authors (Bovie and Hughes, 1918; Hinrichs, 1928; Alpatov and Nastjukova, 1933) found the growth of *Paramoecium* to be stimulated by short exposures to ultra-violet light. It would indeed be rather surprising if strongly absorbed radiation of this type, which is known to affect important biological reactions, did not produce stimulation under some conditions. But it is uncertain how far such effects are to be regarded as genuine physiological reactions. In many cases, as Haffner (1930) remarks, one suspects that they are merely the secondary effects of cell destruction. Probably the irradiation experiments of Reiter and Gabor should be placed in this category, since the onion roots in their experiments at $334\mu\mu$ showed extensive necrosis when exposed for rather longer periods than those required for "positive" effects. A more obvious case of a "stimulation" depending on a local destructive action is the artificial fertilisation of sea-urchin eggs produced by a microscopic beam of intense ultra-violet light (Tschachotin, 1912, 1921). This nevertheless deserves mention, because mitogenetic rays have been claimed to produce the same effect—formation of a fertilisation membrane in unfertilised sea-urchin eggs (Dorfmann and Sarafanow, quoted by Gurwitsch, 1931*c*, p. 230). Beudt (1930), concluded that the "stimulation" of *Rana pipiens* larvae observed by Higgins and Sheard (1926) was only a developmental abnormality due to destructive effects. The observation of Reiter and Gabor, that irradiated *Triton* larvae emerge earlier from their jelly covering than normal larvae, was shown by Beudt to be due only to premature liquefaction of the jelly; he found no sign of stimulation with larvae of *Rana esculenta* and *R. temporaria*. Barbacci (1929), however, did find it with *Bufo vulgaris* larvae subjected to intensities about 1/16 of those used by Beudt. The confirmation of this by Stempell and von Romberg (1932) should be accepted with reserve.

The apparent stimulation of bacteria observed by Browning and Russ (1917) was explained by Coblentz and Fulton (1923–4) and by Gates (1930). (See also Nadson and Philippow, 1928).

To summarise: the work of Chariton, Frank and Kannegiesser has been disputed and most of the remaining claims of stimulation by ultra-violet light, including those of Reiter and Gabor, have either been explained away or have been shown to be due to destructive effects—quite unconnected, therefore, with the present problem. There remain the experiments of Barbacci and their doubtful confirmation by Stempell and von Romberg. But even here the intensities used were enormous by comparison with those of Chariton, Frank and Kannegiesser, and these were themselves enormous by comparison with the mitogenetic rays. There is little justification to be found for the view that anyone has succeeded in producing a mitogenetic effect by artificial irradiation[1].

(4) *Ultra-violet chemiluminescence in liquid systems.*

In general, the quantum energy of the radiation accompanying a chemical reaction cannot exceed the heat of reaction. Now the energy evolved in most biological reactions is rather small: the whole complex process of combustion of glycogen is accompanied by a liberation of only 71·7 Cal. mole. (Brookens, 1933), and the individual stages of this and other reactions which occur are less strongly exothermic: *e.g.*

$$C_6H_{10}O_5 \cdot H_2O \rightarrow C_6H_{12}O_6 - 2 \cdot 1 \text{ Cal.,}$$

hexosediphosphate →lactic acid – phosphate – 15·8 Cal.
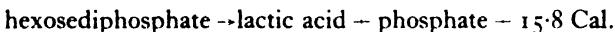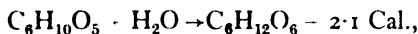
(see Meyerhof and Suranyi, 1927; Meyerhof and Lohmann, 1928). Even if these reactions were accompanied by radiation, which they are not known to be, it would probably lie in the infra-red, with a wave-length about $1\,\mu$. The inorganic reactions which are said to emit mitogenetic radiation have rather larger heats of reaction, *e.g.*

$$Zn + H_2SO_4 \rightarrow ZnSO_4 + H_2 + 34 \cdot 2 \text{ Cal. (corresponding to } \sim 820\,\mu\mu),$$

$$HCl \cdot NaOH \rightarrow NaCl + H_2O + 13 \cdot 7 \text{ Cal. } (\sim 2\,\mu).$$
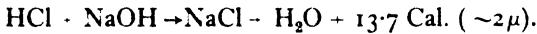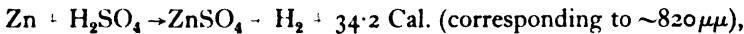
Sometimes the above rule does not apply. The case of ultra-violet chemiluminescence quoted by Gurwitsch is one of the exceptions where the heat of reaction is only 53 Cal. (corresponding to a quantum of green light of wave-length $546\,\mu\mu$) and the luminescence extends as far as $300\,\mu\mu$:

$$Hg + Cl_2 \rightarrow HgCl_2 + 53 \text{ Cal.} \qquad \text{(Haber and Zisch, 1922).}$$

Other cases of the same sort are known. Bonhoeffer (1925) found that the Hg resonance line $254\,\mu\mu$ (110 Cal.) is emitted when hydrogen atoms combine in presence of mercury vapour, although the heat of reaction is only 101 Cal. Bonhoeffer suggested that the excitation energy of the mercury atoms could be accumulated in steps. Another case is the thermal decomposition of ozone, where the radiation

[1] Note added 9th Sept. 1934: This conclusion is strongly supported by the recent paper of Schreiber (1934).

emitted consists of a series of bands corresponding exactly to the emission bands of ozone as determined by Stark (1914), and extending to $232\mu\mu$ (Stuchtey, 1920). The bimolecular reaction $2O_3 \rightarrow 3O_2$ gives only 68·2 Cal. (Jahn, 1908).

Reactions of the type studied by Haber and Zisch have been investigated in detail by Beutler (Beutler and Josephy, 1928; Beutler and Rabinowitsch, 1929-30), who showed that if the sum of the activation energies possessed by two colliding molecules is sufficiently near that corresponding to a higher energy level of one of them, the one partner may be further activated at the expense of the other ("Energiesteigerung"). In living cells most reactions are heterogeneous, and here the frequency of effective collisions between activated reaction products might be enormously increased at enzyme surfaces (cp. Mann, quoted by Haldane, 1931). But most organic molecules would be expected to dissociate or to pass through a predissociation level on receiving the large energies corresponding to quanta of mitogenetic radiation, and would obviously in such a case be unable to emit radiation of this frequency.

A rough calculation from the ordinary kinetic theory collision formula shows that if 1 cm.^{3} of a tissue emits 10^6 quanta per second (assuming an intensity of 10^3 h/sec. and making rough allowance for absorption in the body of the tissue), the energy necessary for these quanta being obtained by binary collisions of 100 per cent. efficiency between activated molecules of molecular weight 100, and the collision radius being 10^{-7} cm., then the concentration of the activated molecules must be about 5.10^{-14} molar at 300° K. This result leaves plenty of latitude for inefficiency of collisions or for the possibility of emission only by a third non-reacting molecule in ternary collision with the two activated molecules.

There is the further possibility, indicated by Frankenburger (1933) and by Fischer (1930), that highly exothermic reactions between radicals may take place as normal stages in certain intracellular reactions—for example, in those enzyme reactions for which chain mechanisms have been suggested (Willstätter, 1932; Weiss, 1934), or those involving hydrogen peroxide. The above calculation applies equally well to cases of this kind and shows that no extravagant assumptions need be made in order to justify, on physical grounds, the existence of a hypothetical chemiluminescence too minute in intensity to be detected by any known physical method.

The literature contains few well-founded statements which suggest that any of these things actually take place in heterogeneous systems. Kalehne (1922) found the air above heated crystalline quinine sulphate to be ionised, and inferred that the ionisation was brought about by radiation. Kreuchen and Bateman (unpublished), however, could detect no radiation with the Geiger-Müller counter. According to Rajewsky (1931 *b*), the coagulation of egg albumen by heat, sulphuric acid, or α-particles from polonium is accompanied by ultra-violet radiation; this has also been disputed by Kreuchen and Bateman (1934). Nor could they find any effect with several of the inorganic reactions which, according to Frank and Rodionow (1931, 1932), emit radiation (see also Kugelmass and McQuarrie, 1924; Sereno and Cruto, 1933).

Thus cases of ultra-violet chemiluminescence in liquid systems are hard to find. Purely physical considerations lead to the conclusion that the phenomenon is possible but rather improbable[1].

IV. FURTHER BIOLOGICAL METHODS OF DETECTING MITOGENETIC RADIATION.

Yeast. At present the most important detector is yeast. Yeast cells growing on solid wort agar media were first used (Baron, 1926; see also Gurwitsch, 1929 b), and the mitogenetic effect showed itself by an increase in the proportion of buds of some fixed size in stained preparations made 1–2 hours after the induction. Baron gives an adequate account of his technical procedure, but his quantitative statements are vague, supported by insufficient protocols. Thus, counts made by different observers agree to "a few per cent."; the percentage of budding cells in different regions of a culture is likewise constant to within "a few per cent." (of what?) if about 1000 cells are counted each time. No true controls are given. Two positive and two negative experiments with onion sole *Brei* as source are given, one with onion root and several with yeast. With a quartz plate between source and detector positive effects were still obtained, with glass they were abolished (one experiment). Baron remarks that the cultures used as detector are not always in "Aufblühen," since the proportion of unsuccessful experiments is "not to be neglected." Sources of error are discussed in more detail by Gurwitsch (1931 c, p. 7 ff.). He presents no definite control experiments, but selects instead from a long series of induction experiments (over 6000) those which "for one reason or another" gave zero effects; from a total of 217, 37 fall between · 0·1 and −0·1 per cent., 183 between - 1·0 and −1·0 per cent., 213 between - 2·0 and −2·0 per cent. It is concluded that differences greater than 2 per cent. indicate a probable induction effect. In other words, experiments which are defined as "unsuccessful" (*i.e.* those giving effects less than + 2·0 per cent. or larger negative effects) are separated from those defined as "successful" (*i.e.* those giving positive effects greater than 2·0 per cent.) and quoted as controls. This statement requires qualification in view of Gurwitsch's claim that the zero effects are confined to experiments of a certain type—for example, that in mitogenetic spectrum analysis (*q.v.*) certain wave-length regions consistently give zero effects, while certain others consistently give positive ones. But how consistently? One recalls Baron's statement that a fair proportion of yeast experiments are bound to be unsuccessful on account of unsuitability of the detector, an unsuitable culture being defined, presumably, as one which did not give a positive result: no other criterion is mentioned (see also the discussions of Blacher and Holzmann (1930 a), and of Frank and Popoff (1930)). Nevertheless Gurwitsch insists (1931 c, p. 13) that no data are accepted unless they are perfectly reproducible. It is hard to know what is meant. As in the case of the onion root method, the published data allow very little valid criticism, but the need for critical repetition of the work is evident.

The time relations in the "Sprossungseffekt" produced by a fixed period of

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[1] Note added 9th Sept. 1934. See Braunstein and Potozky (1934) for a discussion of the same subject from the Gurwitsch standpoint.

irradiation have been studied by Kurajeff (1931). The first effect appears about 30 min. after induction, reaches a maximum after 2 hours, then decreases rapidly, becoming zero at the end of $2\frac{1}{2}$ hours. It is extraordinary that the effect should be so protracted, for the stage of budding actually counted lasts only 15 min. Now if on the average every cell divides once in 2 hours, a continuous positive effect of 30 per cent. lasting over this period can only mean that the effect of 10 min. induction extends over more than one generation of cells.

Nakaidzumi and Schreiber (1931) have examined the yeast agar method. They observed at short intervals the growth of small clumps of induced cells and compared them with control clumps growing under otherwise identical conditions. They found no induction effects, and the normal variation was much larger than that given by Baron and Gurwitsch.

In a later paper Baron (1930) measured the rate at which vacuolised, non-reproducing cells of an old yeast culture recommenced budding on being suspended in a drop of fresh nutrient medium, using a hanging drop technique. Supposing that the cells in such a drop would induce each other[1], and that the degree of induction would be greater with a denser population, he compared the rates of "awakening" of two cell populations of different densities. In a liquid medium, the denser awoke more promptly; in agar there was no difference. Baron imagined beer wort to transmit mitogenetic rays and gelatin to absorb them, so that his experiments appeared to indicate an effect of radiation. Actually both beer wort and gelatin absorb ultra-violet light very strongly (cp. Nakaidzumi and Schreiber, 1931; Kreuchen and Bateman, 1934), and since convection can occur in beer wort but not in agar, a chemical explanation would be more reasonable. The same explanation would fit Baron's claim that dilute cultures (8000–10,000 cells/mm.^{3}) are stimulated under the influence of other mitogenetic sources, while concentrated ones (80,000–100,000 cells/mm.^{3}) are not, though Baron prefers to think that the denser cultures do not act as detectors because they are internally saturated with radiation. The chemical explanation is, however, valueless, if, as Baron asserts later, the same effects are obtained when source and detector are separated by quartz.

The high absorption of beer wort for ultra-violet light ought to make liquid cultures unsuitable as detectors for mitogenetic radiation. It is possible, however, to place too much trust in elementary physics. Even in much larger drops than those used in Baron's experiments yeast in beer wort is an admirable detector. Its use makes it possible to measure the increase in cell population which must necessarily follow an increase in the proportion of buds. Rossmann (1928–9) had previously attempted to observe a macroscopic effect in agar cultures, without success; Baron (1929, 1930) succeeded with his hanging drop technique. Tokin (1933) has confirmed this effect under some conditions, but he denies that it can be obtained when source and detector are chemically isolated from one another.

The development of a reliable technique for induction experiments with liquid yeast cultures has been mainly due to Potozky and Salkind (1931), who used a

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[1] According to Baron, a completely isolated cell divides ultimately only because it can induce itself—i.e. emit and reabsorb its own mitogenetic radiation.

12-hour culture of *Saccharomyces ellipsoideus* in beer wort, containing about 100,000 cells per mm.[3] (*i.e.* ten times the concentration at which, according to Baron, yeast populations become immune from external influences), which was induced in a capillary chamber. Small samples were then pipetted into 0·1 cm.[3] fresh wort and incubated. Counts were made after 1 and 3–5 hours; the error was said to be less than 6 per cent. when about 200 squares of the Thoma-Zeiss chamber were counted, while induction effects were usually greater than 25 per cent. Brainess (see Gurwitsch, 1931*c*, p. 15; Kalendaroff, 1932) estimated the cell population by centrifuging "mycetocrit" tubes. Gurwitsch (1932*a*) quotes 200 "Blindversuche" in which 175 pairs of tubes gave unmeasurable differences in the length of the cell column; the remainder gave differences of 2–6 per cent. Systematic induction effects, on the other hand, amounted to 25–100 per cent.

Richards and Taylor (1932) have made several technical criticisms, and have carried out experiments on dilute cultures of yeast (1000–10,000 cells/mm.[3]) in Williams' medium[1]. The control cultures were in glass tubes, the induced ones in quartz, and both were immersed in suspensions of yeast or bacteria. The percentage of budding cells was determined at 2–3 hour intervals over a period of 12 hours or more. The range of normal variation was in no case exceeded, nor did the actual growth curves differ significantly.

The mycetocrit method has been examined by Kreuchen and Bateman (1934), with care to reproduce all essential experimental procedures described by Gurwitsch. They induced beer-wort cultures of *S. ellipsoideus* in rotating quartz tubes, using yeast-agar cultures, sarcoma *Brei*, blood, onion sole *Brei*, and bacteria as sources of radiation, and having in each experiment ten induced and ten control cultures, instead of the one used by Gurwitsch. The mycetocrit method showed itself to be very reliable, the probable error of the mean for ten single measurements being about 2 per cent. The induction experiments, equivalent to several hundred Gurwitsch experiments, gave no positive effects.

None of the remaining methods of detection are sufficiently consistent to warrant detailed discussion. Reference may be made to the following papers. *Bacteria*: Sewertzowa, 1931; Ács, 1931; Bürgers and Bachmann, 1931; Wolff and Ras, 1931; Harders, 1933; Soru and Brauner, 1932. *Corneal epithelium*: L. Gurwitsch and Anikin, 1928. *Eggs and larvae*: Stempell and v. Romberg, 1932; Wolff and Ras, 1934; Magrou, Magrou and Roubaud, 1931; Vanzetti and Maxia, 1931; Maxia, 1933*a*; Frank and Kurepina, 1930; compare also Magrou, 1931; Magrou and Magrou, 1931; Choucroun, 1930, 1931; Magrou, Magrou and Reiss, 1931; Maxia, 1932*b*.

V. THE INTERRELATION OF MITOGENETIC EFFECT AND CHARACTER OF MITOGENETIC STIMULUS.

Up to this point the discussion has shown that mitogenetic radiation can be studied only, if at all, by biological methods. Information concerning the properties of various sources cannot be obtained by such methods until the detector has been calibrated, so to say, for relationships between the effect produced and intensity of

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[1] Williams, 1920, Richards, 1932.

exciting radiation, period of induction, etc. Here the available information is summarised.

(1) *The effect of varying intensity.*

The intensity of the radiation from an onion root, regarded as a point source, should obey the inverse square law. The mitogenetic effect, however, is in this case independent of distance, up to 38 mm. (Rawin, 1924; A. and N. Gurwitsch, 1924: experiments done in the belief that the beam emitted is parallel!). With a yeast-agar block, which must be a diffuse source, the intensity could be kept *roughly* constant by increasing the area of the radiating surface with the square of the distance. This has been done, and it has been found that the mitogenetic effect depends on the distance between source and detector, and not on the intensity: *e.g.* the effect, strongly positive at 3 mm., becomes zero at 10 mm. (Gurwitsch, 1931 c, p. 253, "Abstandscharakteristik"). The only other information on the effect of intensity is derived from the mitogenetic effect produced by artificial sources. Chariton, Frank and Kannegiesser (1930) obtained roughly the same effect with $6.10^5 \, h\nu/\mathrm{cm.}^2$ sec. as with biological sources ($< 500 \, h\nu/\mathrm{cm.}^2$ sec.). And Frank and Gurwitsch (1927) got practically the same effect with spectral lines of different intensities as with the diffuse light *between* the lines. Reiter and Gabor obtained positive effects after 1 hour's irradiation with a spectral line of intensity several million times greater than the maximum for biological radiation, where one would have expected an exposure of only 10^{-6} sec. to produce mitogenetic depression (*q.v.*) (Rajewsky, 1932). At the other end of the scale, the individual lines of the spectrally dispersed biological radiation still produce much the same effect as the total undispersed radiation (*e.g.* Kannegiesser, 1931). Gurwitsch suggests that the biological radiation is qualitatively different from the artificial, and he develops a theory unconnected with the problem which it attempts to solve (1931 c, pp. 238 ff.)[1].

Two phenomena connected with the effect of intensity deserve mention. One is the so-called "fractionation" effect: the minimum induction time required to produce a positive effect (*q.v.*) can in many cases be reduced to about 1/50 of its normal value if the stimulus is intermittent, with a frequency of about 100 per second (Gurwitsch, 1931 c, pp. 254 ff.). In other cases (*e.g.* with ciliated epithelium), the fractionation has less effect. Gurwitsch attributes this to a natural periodicity of the emitter, supposing that an artificial fractionation imposed upon an already intermittent radiation would have less effect than fractionation of continuous radiation. It is not clear how a sharp periodicity of 100 cycles per second can be produced in a beam with an intensity of only a few hundred quanta/cm.² sec. All effects of fractionation are said to cease if the "dark" periods are less than 0·0005 sec., because each stimulus then falls during the refractory period due to the previous one.

The second phenomenon is this: when source and detector are brought together very gradually the induction effect is zero (Gurwitsch, 1931 c, p. 263; Latmanisowa, 1932).

[1] In spite of this wide independence of mitogenetic effect and intensity, a relationship between the two is nevertheless assumed when convenient (see, *e.g.*, Gurwitsch, 1931 c, p. 42, and Billig, Kannegiesser and Solowjew, 1932).

(2) *The effect of varying induction time.*

As Fig. 3 shows clearly, the minimum induction time necessary for a positive effect to be produced in a given detector ("Zeitschwellenwert," Zsw.) varies enormously for different sources of radiation. It is about 20 min. for the blood of a normal axolotl (Blacher and Holzmann, 1930*b*), and 5 sec. for a spectral line several million times more intense. Clearly the Zsw. is unconnected with intensity.
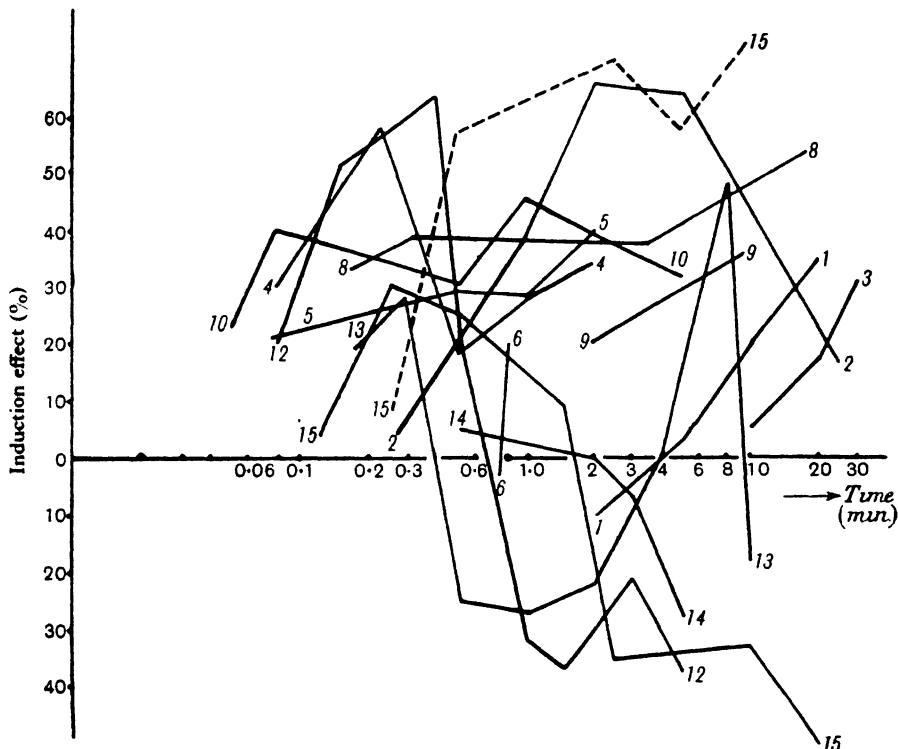
Fig. 3. Relation between mitogenetic response of yeast and period of induction. Abscissa: period of induction, plotted logarithmically. Ordinate: mitogenetic effect, per cent.

Curve 1: tail of tadpole in stage III*b* of metamorphosis (Blacher and Holzmann, 1930).
   ,,   2: same as 1, stage V*b*.
   ,,   3: tadpole blood, stage III*a* (Blacher and Liosner).
   ,,   4: same as 3, stage III*b*.
   ,,   5: blood of axolotl fed on thyroid (Blacher and Liosner).
   ,,   6: ciliated epithelium of gill of *Anodonta* (Zoglina).
   ,,   8: tetanised muscle (Frank).
   ,,   9: *Brei* of resting muscle (Frank and Kreps).
   ,, 10: platinum black and H_2O_2 (Braunstein and Potozky).
   ,, 12: $K_2Cr_2O_7 + FeSO_4$. Showing "mitogenetic depression" (Braunstein and Potozky).
   ,, 13: spectral line 254 $\mu\mu$, "moderate intensity." Shows "periodicity" (Salkind).
   ,, 14: two yeast cultures, fractionated with elliptical sector. "Microeinschleichen" (Gurwitsch).
   ,, 15: comparison of rate of budding and total cell population. Rat's blood (Salkind).

    ----- Total population.   ——— Budding.

The gradient of the induction time—mitogenetic effect curve ("Steilheitscharakteristik") also varies unpredictably with different sources: the effect may reach its maximum with an exposure only a few seconds greater than the Zsw., or it may rise gradually over a period of some minutes. After attainment of this maximum, even the same detector may show qualitatively different types of effect. The early results with onion roots show rather divergent behaviour: according to Rusinoff (1925) and Wagner (1928) the effect is constant for $\frac{1}{2}$–3 hours' and for 2–6 hours' exposure respectively; according to Sussmanowitsch (1928) it becomes strongly negative with 12–26 hours' induction ("mitogenetic exhaustion" or "depression"; cp. A. and L. Gurwitsch, 1928); according to Loos (1930) it reaches a maximum with $1\frac{1}{2}$ hours', becomes zero with $2\frac{1}{4}$ hours', is negative with $5\frac{1}{2}$ hours', and again zero with 10 hours' exposure. With bacteria as detector there are similar contradictions (Sewertzowa, 1931; Wolff and Ras, 1933 b).

Fig. 3 shows some of the phenomena observed with a yeast detector. The negative values found after long periods of induction have been studied by Salkind (1933), who measured both the "Sprossungseffekt" and "Vermehrungseffekt" of the same detector. He distinguished four different types of effect, according to the period of induction:

(1) Sprossungseffekt positive; Vermehrungseffekt zero. Here the increased budding is insufficient to give rise to a measurable increase of population.

(2) Both positive.

(3) Sprossungseffekt negative; Vermehrungseffekt positive. "Apparent depression." No plausible explanation has been given.

(4) Both negative. "True depression."

Salkind shows further that depression may be followed by a second positive, a second negative and a third positive phase (curve 13, Fig. 3, see Gurwitsch, 1931 c, p. 334, footnote).

Evidently the relationship between period of exposure and mitogenetic effect is just as unsatisfactory as that between intensity and mitogenetic effect. Experiments with biological detectors have not yet led to the publication of any intelligible information concerning mitogenetic radiation.

(3) Mitogenetic spectrum analysis.

The success of spectroscopic experiments with mitogenetic radiation (e.g. Reiter and Gabor, 1928; Frank and Popoff, 1930) is astonishing, because the nature of the "Abstandscharakteristik" would seem to destroy the possibility of detecting the radiation at the end of its long path through a spectrometer. Nevertheless, analyses of the radiation from various sources have been described. The method is simple (Kannegiesser, 1931). A long yeast-agar culture is divided by thin celluloid lamellae into compartments, corresponding to 4–6$\mu\mu$ (1$\mu\mu$ in later measurements— Ponomarewa, 1931), and placed in the focal plane of a quartz monochromator. The source of radiation, and a rotating sector, are placed before the collimator slit. After induction, samples from each section are fixed and counted in the usual way. The

result is a mitogenetic spectrum, consisting of bands, $1-3\mu\mu$ in width, which is of a definite type, related to a certain type of chemical process occurring in the source of radiation (see Kannegiesser, 1931; L. Gurwitsch, 1931; Potozky, 1932 a; Ponomarewa, 1931; A. and L. Gurwitsch, 1932 a; Billig, Kannegiesser and Solowjew, 1932; Golischewa, 1933; Klenitzky and Prokofiewa, 1933) such as glycolysis, oxidation, enzymatic splitting of phosphoric esters, etc. The character of the spectra is surprising, and it is difficult to explain why all oxidation reactions, for example, should give spectra of the same type (cp. Potozky, 1932 a). This implies that a closely similar emitter is present in all these reactions, which is perhaps conceivable with the biological reactions (cp. Kautsky and Neitzke, 1925), but incredible in the case of inorganic oxidations.

VI. THE GURWITSCH DEMON: SECONDARY RADIATION AND THE PROPAGATION OF THE MITOGENETIC IMPULSE.

It is at first sight paradoxical that mitogenetic radiation, identical with short-wave ultra-violet light, should pass without appreciable absorption several centimetres along the axis of an onion root, should affect the growth of yeast cells after being absorbed by beer wort, and should yet be prevented from acting by a thin quartz plate which has not previously been tested for "mitogenetic transparency" (cp. Nakaidzumi and Schreiber, 1931; Gurwitsch, 1931 c, p. 203). A simple explanation, however, has been reached by a study of the fact, apparent in the earliest onion root experiments, that the mitogenetic effect spreads longitudinally over the entire meristem, while being sharply limited laterally to a region about 70μ wide. It appears that certain of the cells in the limited area "primarily" induced are stimulated by *absorption* of mitogenetic radiation to *emit* "secondary" radiation, which is in turn absorbed and re-emitted by cells outside the induced region. In this way a mitogenetic impulse is transmitted axially along the root, stimulating mitosis and causing the further emission of radiation. The track of this impulse is miraculously confined to the induced side of the root, and it appears to be incapable of spreading out transversely.

Gurwitsch (1931 b) has observed a similar spreading of the mitogenetic effect in the case of the yeast-agar detector. The effect due to irradiation of a region 0·1 mm. wide extends to 10–12 mm. and actually attains its maximum in the uninduced region. "Wir haben hier ein eklatantes Beispiel der Fortleitung der mitogenetische Erregung" (1931 c, p. 294). In the same paper Gurwitsch states his hypothesis of secondary radiation: "Es wird nun angenommen, dass diese Sekundärstrahler in den Detektorkulturen die Bedeutung von Transformatoren oder Relais haben, indem sie durch minimalen Intensitäten, z. B. durch je *ein* Quant der mitogenetischen Strahlen angeregt werden, aber ihrerseits eine nicht unbeträchtliche Quantenmenge aussenden." The mitogenetic impulse is also transmitted by this mechanism through liquid yeast cultures (Gurwitsch, 1931 c, p. 296), so that the strong absorption of beer wort sets no obstacle to the demonstration of a mitogenetic effect with such cultures.

The experimental evidence for this wave of secondary excitation is briefly the following: (1) If the tip of an onion root is cut off, the stump does not radiate. It can, however, be made to do so by being induced at any point with another root (A. and L. Gurwitsch, 1927; Potozky and Zoglina, 1928). (2) The velocity of transmission of the mitogenetic impulse along the root, measured by the time required for the appearance of secondary radiation at various points, is about 30 metres/sec. (Anna Gurwitsch, 1931). (See also Wolff and Ras, 1933 a.)

Some further phenomena are of interest. Thus the ability of an onion root to produce secondary radiation is somewhat rapidly lost on prolonged irradiation (Potozky and Zoglina, 1928), while yeast-agar cultures set up for "mutoinduction" lose completely their ability to emit mitogenetic radiation (cp. also Latmanisowa, 1932). Gurwitsch attributes these results to exhaustion, by photochemical breakdown, of some substance which is only available in small amounts. Spectral analysis shows that the secondary radiation from the onion root is glycolytic in nature, unlike the primary, which is oxidative[1]. The reaction, then, which provides the energy for secondary radiation is clearly carbohydrate breakdown. This is connected up with the observation of Gesenius (1930 a) that prolonged mitogenetic irradiation of yeast produces a decrease in respiration, which is vaguely explained as "ein Zustand hochgradiger Erschöpfung des Stoffwechsels." It is not clear why cells within a culture do not always exhaust each other by excessive secondary emission, and why any culture can either emit or react to mitogenetic radiation.

The secondary radiation of resting nerve is indistinguishable in intensity and spectral composition from the primary radiation of *excited* nerve. If the radiation actually indicates the chemical reactions occurring in nerve, one would be bound to conclude that a nerve-muscle preparation could be stimulated by mitogenetic radiation (cp. Gurwitsch, 1932 b). Latmanisowa (1932) states that a subthreshold stimulus can be made to stimulate a nerve exposed to mitogenetic radiation.

It is actually claimed in a recent paper (A. and L. Gurwitsch, 1932 b) that mitogenetic radiation can be conducted through a bent tube containing nucleic acid solution: if one end of the tube is irradiated with the copper spark line $322\mu\mu$, the other end is said to emit secondary radiation of wave-length $240-244\mu\mu$. This is a type of fluorescence hitherto unknown.

The physical unreasonableness of the hypothesis of secondary radiation cannot be obscured (cp. Nakaidzumi and Schreiber, 1931). It postulates the presence, in a beer-wort yeast culture, of a demon capable of making an opaque suspension transparent to ultra-violet light. Like Maxwell's demon, the Gurwitsch demon is simply an invention for evading—on paper—a thermodynamical law, with the important difference that we are seriously asked to believe in its existence.

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[1] It is not explained how primary radiation can pass from the onion sole to the root tip: this alleged difference between primary and secondary radiation forces us back to the fictitious transparency of the root for mitogenetic radiation. It is also not explained how secondary radiation can ever be absent from a root which emits primary radiation.

VII. SOURCES OF MITOGENETIC RADIATION.

In 1925 A. and L. Gurwitsch considered the possibility that mitogenetic radiation might originate in the same way as bioluminescence, and, guided by the work of Dubois and of Newton Harvey, they succeeded in obtaining from sole *Brei* two fractions which emitted mitogenetic radiation when mixed. The analogy seeming complete, the active components were christened "mitotin" and "mitotase."

The beauty of the analogy has been marred by later work, because this mode of origin of mitogenetic radiation is far from unique. It has been claimed that the radiation is not a product of any one exceptional reaction, but of certain major metabolic reactions, such as glycolysis, oxidation, proteolysis, splitting of creatine phosphoric acid, etc. (see, *e.g.*, Kalendaroff, 1932). Most of these reactions have given positive results *in vitro*—for example, during the peptic and tryptic digestion of egg yolk, fibrin and dried serum albumin (Karpass and Lanschina, 1929; Kalendaroff, 1932; Billig, Kannegiesser and Solowjew, 1932), during oxidations which occur when oxyhaemoglobin is added to serum or lymph (Sorin, 1926), on addition of glucose solution to dried blood (Kannegiesser, 1931; Gurwitsch, 1933), on addition of thymonucleic acid to carcinoma *Brei* (A. and L. Gurwitsch, 1932*a*), and in the hydrolysis of starch by amylase, or of cane-sugar by saccharase (Klenitzky and Prokofiewa, 1933). Siebert (1928*b*) found that even simpler oxidation models, such as the oxidation of oxalic acid in presence of charcoal, emitted radiation, and Braunstein and Potozky (1932) extended the investigation to inorganic oxidations. These likewise gave positive results. According to Rajewsky (1931*b*) pure aqueous albumin solution emits spontaneously a feeble radiation, the intensity of which is greatly increased during coagulation. Even neutralisation reactions and the simple process of solution of NaCl in water are sources of radiation (Wolff and Ras, 1932*b*, 1933*c*).

Since so many reactions emit radiation, and since the mitogenetic effect is independent of intensity over a wide range, it is surprising to find that in certain tissues, where the intensity of the major metabolic reactions cannot change very greatly, the ability to emit radiation is remarkably sensitive. Thus blood loses its ability to radiate about 10 min. after collection, although the radiation is glycolytic, and glycolysis certainly does not decrease significantly until much later (*e.g.* Lundsgaard, 1933). Blood radiation is said to be a constant phenomenon, persisting even in extreme pathological conditions, but it vanishes after moderate work (Brainess, see Gurwitsch, 1931*c*, p. 132; Latmanisowa, Markowa and Ufland, 1933) and is absent from persons with carcinoma—even at a stage too early for diagnosis (Gurwitsch and Salkind, 1929; Gesenius, 1930*b*; Potozky and Zoglina, 1929). Siebert (1930) says that it returns after X-ray treatment of the tumour. It is of course possible, but highly improbable, that this "extinction" of blood radiation is caused by small amounts of some substance which acts in much the same way as those foreign gases which, added in small amounts, are able to extinguish the fluorescence of mercury vapour (cp. Braunstein and Heyfetz, 1933).

Again, stimulated muscle is said (Frank and Popoff, 1930) to radiate only during the latent period, which according to Roos (1932) lasts less than 0.4σ; the production of radiation has certainly stopped within 12σ of stimulation. It is surprising that the subsequent processes do not produce any radiation.

Stimulated frog's skeletal muscle invariably gives positive results, and never "mitogenetic depression"; but heart muscle presents "ein exquisites Gegenstück" (Gurwitsch, 1931 c, p. 155), giving positive and negative results with almost equal frequency. Gurwitsch adds that positive effects are most frequently produced by exposure to 40, negative by exposure to 60 beats. The case of nerve also presents an instructive anomaly. A single nerve impulse is accompanied by radiation (Wassiliew, Frank and Goldenberg, 1931), the spectral composition of which differs according to the method of excitation (Kalendaroff, 1932); the point at which two opposite impulses meet does not, however, radiate (Schamarina, 1932; cp. A. V. Hill, 1933; Gurwitsch, 1933 b), in accordance with Schamarina's expectations, which were based on an erroneous interpretation of the phenomenon[1].

VIII. CONCLUSION AND SUMMARY.

This article began with a brief account of the theoretical views which led to the first mitogenetic experiments. They were unconvincing, but since erroneous theories occasionally lead to important discoveries, it seemed desirable to examine the experiments carefully. Considered with the various attempts that have been made to repeat them, they were not quite satisfactory, but statements made as to the reproducibility of the phenomena in question obviously could not be contradicted. There is insufficient experimental evidence to refute them with any certainty. Admitting for the time being the correctness of these fundamental experiments, the proof that the effects said to be observed were due to radiation was even less satisfactory. Granting again that they were nevertheless due to radiation, it was shown to be impossible to arrive at the conclusion that the radiation is identical with short wave ultra-violet light without ignoring some of the experimental data. Even when, for the sake of argument, one did this, one found a discrepancy of about $100\mu\mu$ between the values found by different observers. The examination of supposed sources of mitogenetic radiation by physical methods, still assuming its identity with ultra-violet radiation, led to the result that probably the intensity is too small for the radiation to be detected by any known physical method. Such methods being useless, it was necessary to return to the more sensitive biological methods and to find whether a physically intelligible idea of the mitogenetic effect could be arrived at. There appeared, however, to be no rational relationship between the effect and the stimulus producing it, and the phenomena described often seemed free from the limitations imposed by ordinary physical theory.

In reading mitogenetic literature it is almost always necessary to make extravagant concessions in order to dispose of discrepancies which should have received experimental resolution; the phenomena display a capriciousness which admirably

[1] For a more systematic account of the sources of mitogenetic radiation see Gurwitsch (1931 c).

qualifies mitogenetic radiation for its role of "universal factor" but which is scientifically disconcerting. It is useless for the already complicated analysis of the mitogenetic effect to be carried further without a preliminary attempt to remove the earlier paradoxes.

To sum up: The unsatisfactory state of mitogenetic literature makes it advisable to regard all that has been written in support of the existence of mitogenetic radiation with the greatest scepticism. The existence of a mitogenetic effect has been neither finally proved nor finally disproved; the evidence against it, though extensive, is as yet insufficient. But supposing that a mitogenetic effect does exist, it is highly improbable that it has anything at all to do with ultra-violet radiation.

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UTRICULARIA

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CONTENTS.

THE genus *Utricularia*, including *Polypompholyx* and *Biovularia*, has a claim to uniqueness second to no other group of plants though some may place the orchids on a par with it. Because of the generally small size of its representatives (there are a few notable exceptions, *e.g. U. reniformis*, *U. montana*, etc., which have found their way into greenhouses) the genus will scarcely share even the superficial interest given to orchids, and deeper concern with it will be largely confined to those of more penetrating enquiry. Their size is against them: had the bladderworts been more imposing in this respect they would long ago have yielded their secrets to the cupidity of curiosity. As it is, it is only recently that something like a comprehensive view of the *ca.* 240 species has been afforded. The following is a summary of our resulting knowledge, aside from the purely taxonomic and formally morphologic.

I. THE EMBRYOLOGICAL PERIOD.

What we know of the biology of the embryo, including germination, we owe chiefly to Merz, Lang and Merl, who worked in Goebel's laboratory, following the work of Kamienski (1877), Warming (1874) and Goebel (1891). The material available did not permit a thoroughgoing examination of the whole course of events in detail. It emerged, however, that *Utricularia* and *Polypompholyx* display some of

the physiological-anatomical features related to the nutrition of the embryo first noted by the truly great observer Hofmeister, whose work pointed the way to those modern studies to which impetus was given by Goebel, in which the physiology of the embryo rather than its merely anatomical-morphological course of development, typified in the work of Hanstein, is enquired into. Even now, that chapter of botany remains but meagrely surveyed.

Several species of *Utricularia* were examined by Merz and by Merl (1915). They offered cytological and anatomical evidence that there develops in the chalaza, around the antipodal end of the embryo sac, a mass of nutritive tissue, which doubtless serves as a source of food supply, not unlikely elaborated by it from materials afforded by the vascular tissue reaching the chalaza. A similar island of tissue develops meanwhile in the placenta, placed opposite the micropyle of the anatropous ovule. As the embryo sac grows, it thrusts forth its egg-apparatus end through the micropyle, penetrates the placental epidermis and a haustorial behaviour becomes apparent. An accumulation of transitory starch in the haustorium, whereas none is evident in the nutritive tissue in contact with it, is evidence that there has been a transfer of soluble nutritive matter (sugars). As the embryo matures, cells of a nutritive character appear in its epidermis (and more or less just below it) of its basal end, in contact with the placental nutritive tissue. The meagre endosperm, definitively becoming two separate masses between which a specialised mid-region of the endosperm pinches off the basal part of the embryo, which is then lost to it (Merz), partly, at least, accounts for the absence of root in the definitive embryo. Remains of the upper mass of endosperm can often be observed when young seedlings are freed from the testa. Further study of these relations is required.

Lang examined *Polypompholyx*. During the development of the embryo there is an extensive and complex haustorium formed from the suspensor (?) which withdraws nutriment from the endosperm, recalling, for example, the case of the Rubiaceae (Lloyd, 1902).

II. THE DEFINITIVE EMBRYO.

In most cases the fully developed embryo consists of an oval (*U. orbiculata*, *U. monanthos*) or subspherical, often longitudinally compressed, mass of parenchyma with little differentiation, sufficient only to make evident the epidermis. There is not the slightest evidence of a root (as occurs in some instances elsewhere), nor is a root ever developed adventitiously by the embryo or by the subsequent plant. The other pole of the embryo may definitively be (*a*) merely a smooth dome of tissue with no further differentiation (*U. lateriflora, U. monanthos*) or, more rarely, (*b*) it may have two cotyledon-like outgrowths of slight development (*U. orbiculata* (Kamienski, 1876), *U. exoleta* (Goebel, Merz)), or (*c*) there may be a corona (pseudo-whorl) of low ill-developed protrusions (*U. vulgaris, U. stellaris, U. inflata*) (Kamienski (1876), Merz) which in some cases continue their development until considerable size is attained (*U. reniformis, U. nelumbiifolia*). The claim that these are arranged in a definite phyllotaxy (Kamienski) is of doubtful validity. In the tropical *U. nelumbiifolia* germination proceeds without rest, affording an example of vivipary (Goebel).

In all cases the embryo is heavily loaded with starch. It is characteristic that it is encapsulated with a faceted integument derived from the epidermis of the ovule. Each facet (caused by the compact crowding of the developing ovules on the fleshy rounded placenta) is ridged or fenestrated in a variety of ways.

III. GERMINATION.

Whether the embryo has or has not any differentiation of cotyledonoids, there are recognised two (or there may be three) types of germination: (*a*) that in which two cotyledonoids arise from lateral positions below the dome or apex of the embryo (*U. lateriflora, U. capensis, U. Lloydii*). One of these is of limited growth and becomes leaf-like with a more or less expanded green blade with stomata; the other becomes a stolon, is positively geotropous and so penetrates into the substrate. Its heliotropy is only slight and is not, as Goebel supposed, the dominant factor in determining direction of growth. In *U. montana*, according to Goebel (1891), a leaf and a trap arise, the latter instead of a stolon. (*b*) On the view that there are three types, *U. monanthos* may be regarded as exemplifying the second (Fig. 1). In this the whole apical dome of the embryo develops into a leaf. From the cylindrical base of this arises a stolon at some distance from the top of the seed, and from the axil (dorsal) of the leaf on the stolon arise three buds. During germination the whole upper half of the embryo undergoes elongation. One of the lateral buds becomes a trap, the fate of the others being variable but usually a second leaf appears. Whatever morphological interpretation may be given to this arrangement, it is evident that the previous type, at least as represented by *U. capensis*, may be assimilated to that of *U. monanthos*. (*c*) That in which the two to about ten cotyledonoids develop as leaves of primary form surrounding the apex of the embryo on which in lateral positions arise one or three lateral members, namely a stolen (*U. exoleta*, Goebel) or a stolon and two bladders, or better traps (Warming, Kamienski), their entrances facing inwardly and therefore ventral.

In the first, (*a*) above, one of the organs first produced becomes a leaf in function, green and furnished with stomata[1] (sometimes if not always, even when wholly submerged, as in *U. caerulea*), its functionally upper surface ventral. In form it is usually more or less expanded. The other organ remains cylindrical, tapering, penetrating the substrate, and quickly produces traps which attain a relatively large size, so that the young plant is quickly supplied with these organs which are, moreover, quite functional. In the second, (*b*) above, the cotyledonoids are all leaf-like and of limited growth, as are of course the traps, while the stolon is a leaf-bearing shoot of unlimited growth. In some the primary leaves (cotyledonoids) are acicular to more or less incised (*U. vulgaris*), in others they have expanded blades (*U. reni-formis, U. nelumbiifolia*, etc.) (Warming, Kamienski, Merl), all of a juvenile form.

The further course of development may now be of two types, (*a*) abrupt or (*b*) diffuse. (*a*) When abrupt, a radially symmetrical true shoot arises from the apex proper of the embryo (as in *U. bifida, ogmosperma, violacea*, in Goebel's view),

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[1] *U. bifida* would produce stomata only when not submersed (Goebel, 1891).
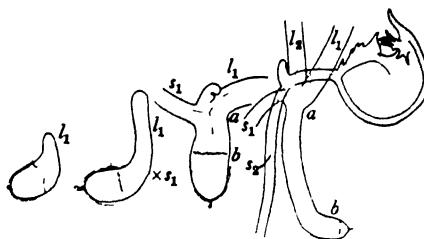
Fig 1
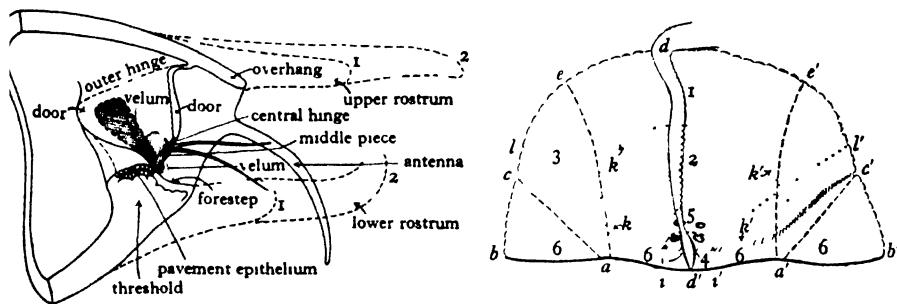
Fig. 2
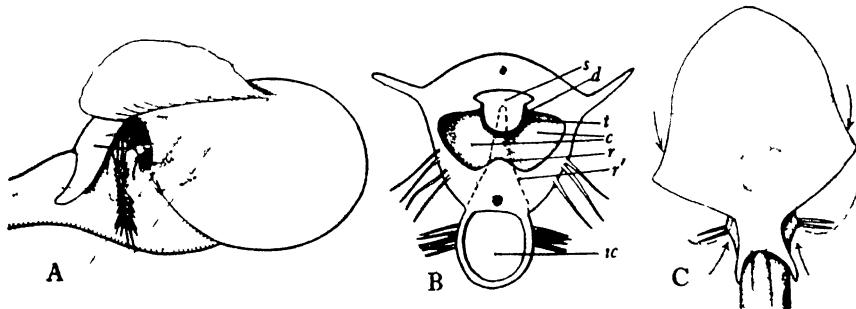
Fig. 3

Fig. 1. Germination forms of *U monanthos* *l*, leaf, *s*, stolon, *a–b*, elongated part of seed and base of leaf.

Fig. 2. Key to the general terminology used Above, sagittal section of entrance structures; 1, 2, lesser and greater extensions of upper and lower rostrum On right, door *bdb'*, line of attachment of outer hinge; *bd'b'*, free edge of door: *ad'a'*, middle reach; *ab* and *a b*, the lateral reaches, areas 6 lie against the threshold, area 1, 3, 6, outer hinge, area 5, middle hinge, area 4, bounded by broken line *kk'*, middle piece of door; dotted line *kk'* separates middle area of door from the outer hinge For key to threshold see Figs. 4 (5), 19.

Fig. 3. A, lateral view of *Polypompholyx*. The lateral wing is shown as turned up. B, transverse section as indicated by the broken lines in A. *s*, space above the door giving entrance to the interior of the trap, *d*, door resting on the threshold *t*, *c*, antechamber leading to the entrance proper, *r*, ridge of the stalk, the portion below the overhang projected and indicated by broken line; *ic*, intercellular canal. C, trap seen from above; the arrows indicate the direction of approach to the entrance

or from the base of one of the two cotyledonoids.[1] This quickly expands to form a conical base of a scape. On this base only anchoring stolons and leaves are produced—never extending, branching stolons—and there is no multiplication of individuals vegetatively. Such plants are annuals and evanescent; their development is so brief that one may usually observe the original embryo with integument and its embryonic structures still adherent at the base of the scape (*Polypompholyx*). (*b*1) When the base of the scape produces branching stolons, these may be more or less limited in their extension and the plant may still be an annual (*U. Lloydii*), but the course of development may be described as diffuse. The same question as to the exact origin of the scape arises. In any event, no further scapes are produced beyond the primary one. (*b*2) Instead of the scape springing directly from the embryo, its production is delayed until a more or less extensive mat of stolons has grown. To this type of development belong on the one hand many anchored forms (*U. orbiculata, U. caerulea,* etc.), and on the other the floating and semi-floating kinds (*U. vulgaris, U. intermedia; U. tubulata* should be studied) and the anchored massive forms, *U. reniformis, U. Humboldtii,* etc. In these the scapes arise from the upper (ventral) surface of stolons, but in the anchored forms their origin is often obscured by a plenitude of secondary organs, while the original stolon is readily broken off unless very great care is used in dissecting out the plants. These plants are perennial or are carried over during winter by winter buds (turios) (*U. vulgaris,* etc.).

Whatever the type of germination, the floral axis gives rise at its base to secondary lateral organs (leaves, stolons) with specific differences. The positions of origin of these organs appear not to conform to a formal plan. In the submersed floating species, the stolons bear (*a*) two ranks of leaves laterally, with, in some species, a series of "airshoots" bearing only small bracts from the upper surface: the airshoots, though submersed, are supposed to have some such significance as indicated in the name; or (*b*) in many other anchored species a single lateral rank of leaves accompanied by a corresponding rank of root-like (in behaviour) stolons. The leaves are, at least in some cases, set with their blades *in the plane* of the stolon (*U. caerulea*). In *U. orbiculata* (Goebel, and many others) they face *backward,* that is the ventral surface is directed away from the apex of the stolon on which it is borne, but *toward the original seminal axis,* with axillary buds correspondingly in the lower axils. It may be questioned whether the leaves are not variously placed, rather than conforming to a rigid rule. The apices of the growing points are either in-rolled or straight.

Traps occur transversely placed on the stolons in leaf positions, or on the leaves on the dorsal surface, or near the angles of the dissected leaves of floaters, and face usually with the entrance toward the *apex vegetationis* of the stolon. Experiments in rejuvenation (Merl) have shown that traps may arise from rejuvenated tissues.

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[1] The evidence before me (*Polypompholyx, U. violacea*) indicates the former to be the correct interpretation, but this requires confirmation or rebuttal. The case of *U. bifida* is open to further scrutiny.

IV. THE LEAF.

(1) FOLIAGE.

The leaf is generally much restricted in variety of form. The terrestrial types have (with a few notable exceptions) small acicular, terete, or linear to spatulate leaves, rarely orbicular, and in one (?) species (*U. peltata*) definitively peltate—a very rare condition. While frequently very small or minute, the leaves of some species, such as those growing in shallow water, are larger, thin, elongated oval (*U. equisetecaulis* Blat. and McC.). A few species, perhaps mostly those in the affinity of *U. orbiculata*, and some Australian kinds, do not bear leaf traps, but the classification of Goebel (1891) has limited usefulness.

The notable exceptions are such species as *U. reniformis*, *U. nelumbiifolia*, *U. longifolia*, etc., having large, indurated leaves, which, aided by large and showy flowers, give them the aspect of orchids. These species are apparently the most terrestrial of all, whose stolons and bladders are, however, equally confined to a watery environment. In some cases (*U. Humboldtii*) there are submersed dissected leaves (it lives in the water held by Tillandsias (Gardner, 1846; Ule, 1898)) and indurated entire air leaves with elaborate anatomy.

In the floating submersed species the leaves (*a*) are dissected little (*U. exoleta*, *U. emarginata*) or much (*U. vulgaris*) according to species, or (*b*) are absent, the plant consisting of whorls of branches each ending in a bladder (*U. purpurea*, etc.; *Biovularia* ?). Among the forms (*a*) above there are species which grow more or less anchored in mud or in matted just-submersed vegetation. In these the leaves lie at the water surface or may project just above ("land forms," Glück); here they develop less in length and more in breadth and with reduced dissection, *e.g.* *U. intermedia*, and in these forms there is usually dimorphism of the stolons, some penetrating the substratum (mud) and bearing mere vestiges of leaves, though the bladders are prominent. Stomata occur commonly.

The biologically more interesting features of leaf and stolon are the adaptational structures described as floats. It is well known that all species have extensive, gas-filled, intercellular space systems (lacunae) which levitate floaters but are likewise present in anchored forms, present but less extensive in such as *U. reniformis* (Hovelacque). In some species these spaces are locally much enlarged by the hypertrophy of the leaf axes. Leaves thus modified occur in whorls near the base of a flower scape and serve to float it (*U. stellaris*, *U. inflata*). Or, instead, the basal portion of the floral axis may be inflated into a "spar-buoy" as described by Merl in *U. Warmingii*, and which occurs also in *U. tubulata*, *U. purpurea* and perhaps others. (F. v. Mueller, see Lloyd (1934)).

(2) THE STOLON.

Another condition of leaf modification is found on certain stolons arising from the base of the scape in the axils of bracts (Goebel). They may consist of short, fusiform, simple or compound appendages densely clothed with mucilage glands

(*U. affinis*, Goebel; *U. Lloydii*, Merl), or the dissected leaf may develop as short, bird-claw-like structures ("*Krallen*") (Glück, 1906). The function of these structures is unknown. Most obvious is the suggestion that they (the *Krallen*) are capturing or anchoring mechanisms, but examination fails to show either to be true, even less so for the fusiform structures, though this does not mean that the stolons themselves do not serve for anchorages. In *U. caerulea* these stolons, which produce the short glandular branches, grow to a considerable length, increasing in diameter and bearing short branches and bladders. They may arise in other positions than from the scape bases, but such places may be the initial stages of development leading to the formation of scapes. Stolons bearing these specialised appendages do not occur in, for example, *U. monanthos dichotoma*.

In *U. subulata* each scape base produces one (or more?) very large and far extending naked stolon, the cells of which are loaded with starch. The function of these organs has not yet been fully determined, but in some cases they give rise terminally to new scapes.

In two species (*U. neottioides* (Brazil) and *U. rigida* (West Africa)) which grow in running water, certain stolons anchor the plants by adhering to the hard substratum (rock surfaces, etc.). They, like their analogues in the genus *Podostemon*, are algal in appearance and texture, recalling the holdfasts of some marine forms (von Luetzelburg). While these species are obviously related to the freely floating type *U. vulgaris*, their chief axes are radial and quite stiff, evidently helping to raise the inflorescences above the surface of moving water.

In a few species (*e.g. U. nelumbiifolia*) some stolons become stout aerial affairs, reaching from one water-filled Bromeliad rosette (in which the plant lives) to another (Sra. de Orgao, Brazil; Gardner, 1846).

(3) TUBERS.

Tuberisation of the stolons occurs in some species. Minute spherical or oval tubers resembling a string of beads were described by Goebel for *U. orbiculata* collected by him "in Ceylon[1]" and for *U. brachiata* (Compton), and regarded as water storage organs. These species live in wet moss on tree trunks, rock surfaces, where it may be inferred there is a danger of drying out.

Among other South American species, *U. montana* has spindle-shaped tubers arising with rosettes of leaves and appearing on strong spreading stolons, and Darwin (1875) satisfied himself experimentally that they are water storage organs. His work seems conclusive.

The Australasian *U. Menziesii*, which has no running stolons, produces several oval tubers at the base of the radial scapose axis. It occurs in shallow water or wet soil subject to drought (Goebel).

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[1] It is curious that this plant seems never to have been collected by anyone else. I have studied Goebel's material of this supposed species and the affinities which, in regard to the tubers, differs from all the herbarium material I have seen (Kew, British Museum).

(4) RESTING OR WINTER BUDS (TURIOS).

Certain species, at least many of those in temperate climes, suffer, towards the end of the growing period, a sudden reduction of internodal growth near the *apex vegetationis*, so that the leaves, which also assume reduced forms, become crowded. Thus is formed a tightly packed bud (Crouan Frères, 1858) which frequently sinks to the bottom but may remain floating and become frozen in by ice (Benjamin, 1848; Clarke and Gurney, 1920). These subsequently proliferate from the leaves (only ?) and produce new plants. Such structures are conspicuous in *U. vulgaris*, *U. intermedia*, etc., but are absent from *U. gibba*, *U. emarginata*, and the like.

V. TYPES OF TRAPS.

In what follows will be presented the major details of a series of types, giving in each case examples of the species known by me to conform to the type. Within such groups there is some degree of variety, to be indicated when important.

Traps occur in leaf positions or in some relation to the leaf, but in all cases they are morphologically leaf equivalent.

From the point of view of development the traps were studied quite thoroughly by Meierhofer (1902). For our purpose a simple statement may be made to the effect that, allowing its attachment by its stalk, the trap proper develops in the fashion of a gastrula of two continuous layers of cells (outer and inner epidermis) with a discontinuous mesoderm which persists locally, especially about the entrance, giving a high degree of mechanical strength in this position. The door and threshold arise as inturned lips of the entrance. The various differences characteristic of different traps arise late in the course of development.

VI. THE TRAP OR BLADDER.

In order that the reader may avoid confusion during the perusal of the descriptions to be given below, the diagram, fully labelled, is given (Fig. 2).

We have here to do with the general characters of the trap aside from the structure in particular of the entrance mechanism. Of the former there is an accumulated mass of information, observations of all the observers elsewhere mentioned, as also of many taxonomists who have made use of the readily observed characters of the "bladder" for the completer diagnosis of the species described by them. No taxonomist has outdone Otto Stapf in this exercise, the clearness and detail of whose descriptions are without rival. At best, however, the treatment of the trap leaves generally much to be desired.

The trap is always a small hollow, laterally compressed organ, with walls generally of two courses of cells in thickness except in special regions, particularly where, just within the entrance, there is a semicircular collar (Darwin) or threshold of much more rigidity than the rest of the whole structure. In *Polypompholyx*, however, the trap is triangular in transverse section (Fig. 3). The region above the entrance is sometimes stiffened by additional cell rows (*U. amethystina*). *Polypompholyx* is

composed of at least four layers of cells and the walls are correspondingly massive (Lang). At the other extreme, there is but little increase in thickness of the wall, even beneath the threshold (*U. purpurea*), and the whole system is of great delicacy of construction. In size the traps range from 0·4 mm. or even slightly less (*U. resupinata*) to 3 mm. or in exceptional cases even to 5 mm., the measurement being that of the greatest diameter aside from the appendages (*U. diploglossa*).

The sagittal line of the trap is traversed by a single strand of vascular tissue emerging from the stalk, branching to send one arm in one direction, the other eventually as far as the threshold where it again branches, each branch extending up one side of it. Usually composed of phloem only, it may also have a single xylem vessel (*e.g. U. monanthos*).

Apart from special appendages in the form of protuberances or peculiar trichomes, the outer surface is always studded with small, sessile, mucilage-secreting trichomes, consisting of a basal wedge-shaped cell buried in the outer course of the wall, surmounted by a thin disc-shaped cell with cutinised walls, supporting a capital of a globular single cell (sometimes cylindrical or conical) or two closely compressed cells, free of the cuticle which breaks away early. In the group represented by *U. purpurea* there are three kinds of surface trichomes distinguishable by their capitals, those with rather long cylindrical cells, those with two pyriform cells enclosed in a common loose cuticle and secreting an oil, and those with the capital cell similar to those in general above described (Lloyd, 1932). In all cases these trichomes are common to the trap and the general plant surface, and it seems impossible to assign to them a special function in the trap, where, however, as elsewhere, they may play a part in the excretion of water, which in the trap may result in the reduction of water pressure within (on this see Czaja, 1922 a).

The interior surface of the trap is clothed with a crowded pile or a sparse scattering of trichomes of construction similar to the above, but with the capital cells elongated into a single long cylindrical member or transversely into long arms, two ("bifids") or four ("quadrifids," Darwin) (Figs. 13, 14). Very numerous in some species (*U. vulgaris*, etc.), they are very few in others (only four bifids and four quadrifids, *e.g. U. longiciliata*). There is always the following relation: if the general inner surface is clothed with quadrifids, those trichomes found on the inner wall surface is clothed with quadrifids, those trichomes found on the inner wall surface of the threshold are bifids or also singles; if bifids (*e.g. U. caerulea*), on the threshold they are single. In many cases of the four arms of quadrifids, two may be directed more or less obliquely with relation to the other pair (Meierhofer), and such differences sometimes serve to distinguish species.

The more special features are those found in the form of the entrance and the appendages (antennae, rostrum, wings, etc.) which give character to that general region, or in their absence. The last-named condition is rare and is found in only three New World species, *U. cornuta* (Schimper), *U. juncea* and *U. nana*. The trap is entirely naked, the entrance being overhung (except in *U. nana*) by a somewhat extended beak which is an extension of the hollow interior over and beyond the general plane of the opening (Fig. 4). Growing in the wet borders of swamps with

its stolons and traps buried in a more or less muddy substrate, the traps are, as it has been argued, adapted to such an environment since antennae would have no use. The presence of antennae, etc., in other species in similar habitats (*U. intermedia, U. gibba*) hinders the acceptance of the argument. In *U. resupinata* (Fig. 5), however, the traps are, as in several other species, of two kinds—those on the leaves and exposed to the water, and those on the lower parts of the leaves and on the stolons, buried in the substrate, the plant growing in shallow water but anchored. The water traps are supplied with appendages (antennae) like those of *U. vulgaris*, while the buried ones have very much reduced antennae with very few and reduced trichomes, thus showing that there is a tendency toward the loss of complicated and delicate appendages from buried traps (Lloyd). The general question of the usefulness of appendages is one open to investigation, the teleological importance of them having been too easily imagined—witness the efficiency of a trap having no such appendages. Sceptical views, however, are not wanting.

Next in simplicity is the type of *U. purpurea* (Fig. 25), in which the lower lip of the entrance is quite simple or is prolonged forward into a single, upcurved, proboscis-like extension, armed with a few tapering trichomes, supplemented in some species with two clusters of similar trichomes, one on each side above the entrance (v. Luetzelburg, Goebel) (Fig. 6). In *U. longeciliata* (N.W.) the lower lip is thickened and enlarged into a rostrum bearing two laterally extended branches, while the upper lip is extended as a small beak. There are no trichomes (Merl). This is a trap of the smallest size (0·2–0·3 mm. long) (Fig. 7).

In *U. lateriflora* (Austr.) (Fig. 8) the upper edge of the entrance bears a strong, usually downwardly bent, naked proboscis (upper rostrum). Leading obliquely upward toward the lower lip of the door and on each side is a comb of short and stout trichomes with round capitals (Kamienski, 1876); while a nearly related Bornean *calliphysa* (one other species (?) in Ceylon) has the same combs with elongated cylindrical capitals, and with an additional low row of tubercles extending to each of the upper angles of the door (Stapf) (Fig. 9). In the Himalayas (Sikkim) there is a species, *U. multicaulis* (Fig. 10), described by Oliver, which, like *U. lateriflora*, has a single central extension, but in this case it is expanded into a fan-shaped structure with six radiating multicellular glandular arms, somewhat as in *U. Welwitschii* and *U. firmula* (Fig. 11). On examining material sent by Hooker (and collected by him) to Darwin (1858), the latter incorrectly interpreted the fan-shaped extension as a pair of broadened antennae[1]. In other, Australasian, species there is a similar beak with a pair of wing-like appendages extending to the upper angles of the door, and with another (ventral) pair leading to its lower edge (*U. monanthos*) (Merl) (Fig. 12). Among the floating forms there is one species at least (*U. tubulata*) with a very long beak and two lateral extensions of similar shape and length, affording a unique example (Lloyd, 1934) (Fig. 35). The peculiar Australasian genus, *Polypompholyx* (Fig. 3), has a broad beak, splitting into two short and broad antennae

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[1] Dried material kindly supplied by the Herbarium of the Natural History Museum, South Kensington, of Hooker's original material did not allow an exact enough view of the door structure but the threshold is of the *U. caerulea* type. The position of the stalk and general form, however, do not conform to *U. orbiculata* as Oliver thought.
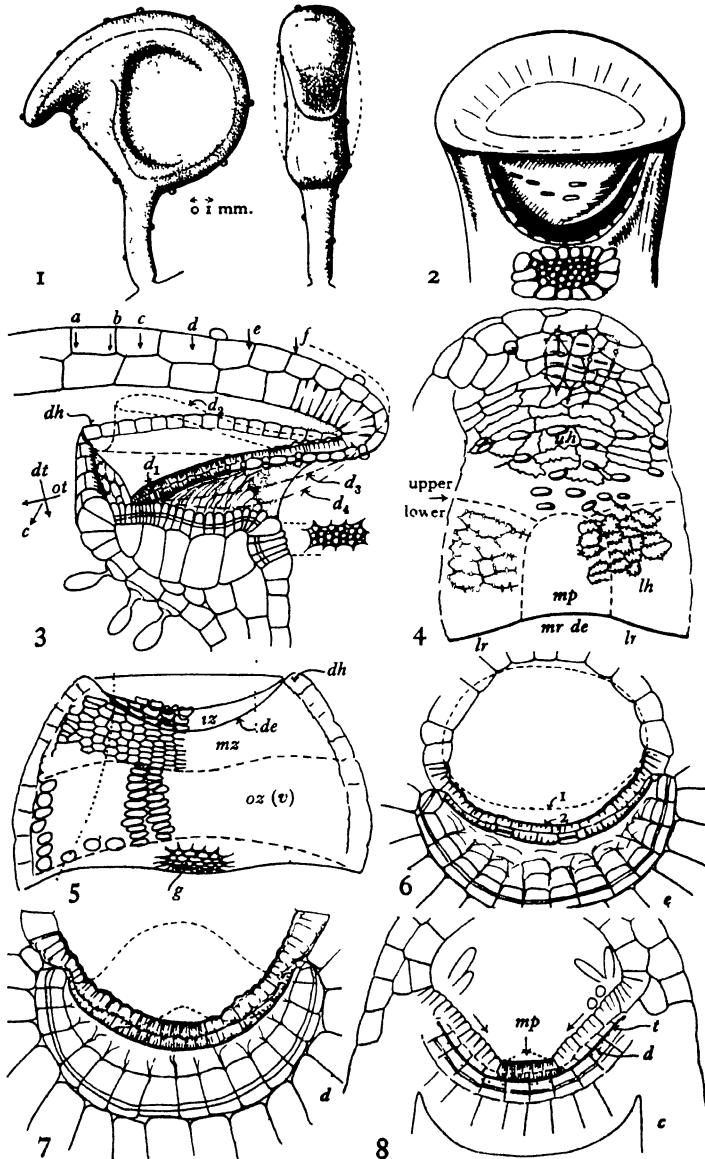
Fig. 4

U. *cornuta*. (1) side and front views of the trap. (2) front view of entrance, lure **glands** below. (3) sagittal section of entrance with the door in the set posture; its relaxed postures **indicated** by d_3 and d_4, its open condition by d_2; the direction of thrust of the lateral hinge (dt) and the **longitudinal** thrust of the door (ot) together with their component (c) are indicated. (4) view of **the door** as from beneath; mp, middle piece; lh, lateral hinge; uh, outer hinge; $mrde$, middle reach of **door** edge; lr, lateral reaches. Cells shown in lighter outline are those of the inner course. (5) dh, de, **threshold**; iz, inner, mz, middle and oz (v), outer zones, the last bearing the velum; g, lure gland. **(6)** transverse section of the door in (1) set and (2) relaxed posture, through (3) e above. (7) same **through** (3) d above, the broken lines indicating partial and full open postures. (8) same through (3) c above; mp, middle piece; t, rear edge of threshold; d, the dotted line indicates the edge of the door; **pointers** show the direction of thrust of the lateral areas of the outer hinge on the middle piece.
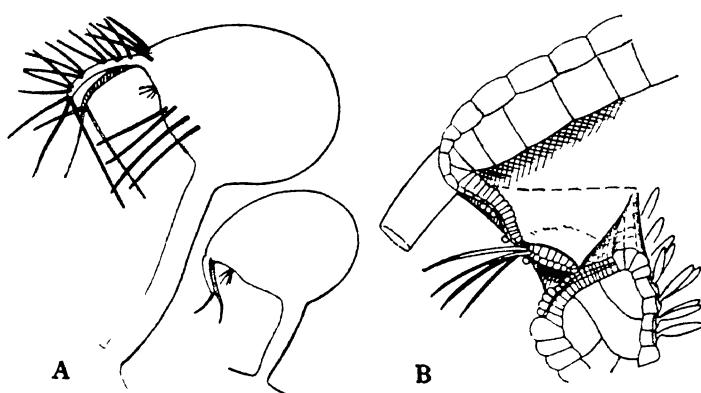
Fig. 5
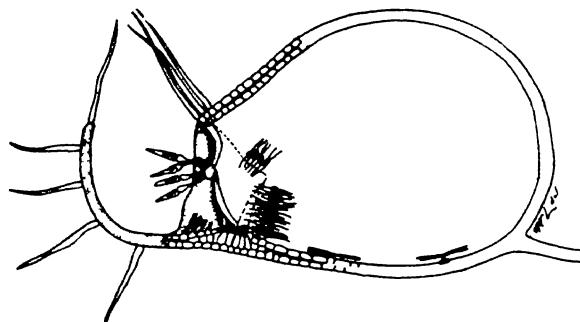
Fig. 6
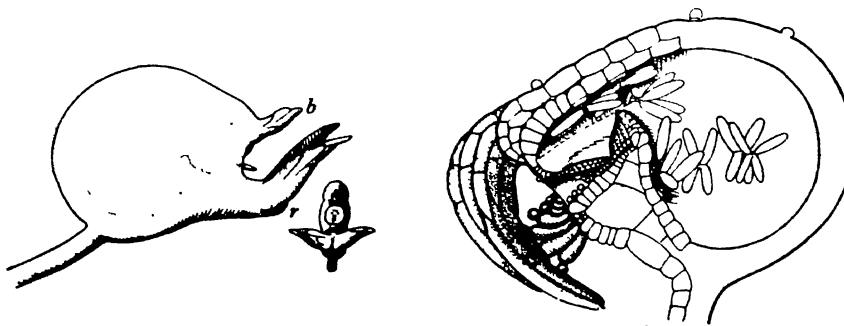
Fig. 7

Fig. 8

Fig. 5. A, lateral views of trap of *U. resupinata*. B, sagittal section of the entrance.
Fig. 6. *U. elephas* (?).
Fig. 7. *U. longeciliata*.
Fig. 8. *U. lateriflora*.
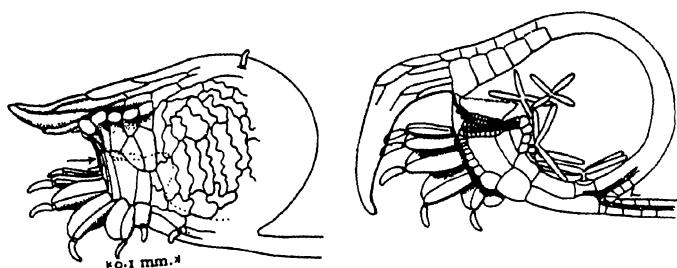
Fig. 9
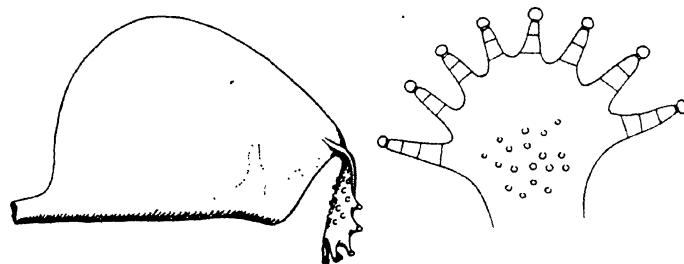
Fig. 10
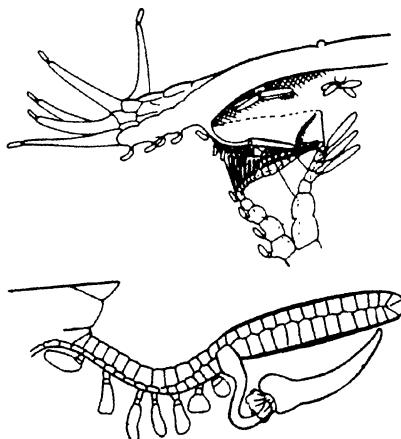
Fig. 11

Fig. 9. *U. calliphysa* (probably).
Fig. 10. *U. multicaulis*.
Fig. 11. *U. Welwitschii*.

which more or less clasp the much-inflated stalk, with its sides extended laterally into two downward-lying wings, while the upper side of the stalk beneath the beak is flattened into a curved ridge, making the entrance a double one (Lang). The wings and stalk partition bear long slender trichomes, as also the floor of the entrance, all leaning toward and pointing at the central area of the door and forming a pair of conical approaches thereto. A band of trichomes placed transversely on each side of the stalk prevents approach of prey from in front.

There is a large number of species, chiefly if not all anchored, otherwise not at all uniform in structure and found in the New and Old World, in which the appendages consist solely of quite simple and naked tapering antennae, generally of some length and extending with graceful curves forward (*U. caerulea, U. Dusenii*) (Fig. 29), or downwardly re-curved (*U. reniformis, U. Gibbsiae*). These antennae arise from the edge of a portion of the wall reaching forward beyond the insertion of the door, and which may be conveniently termed the overhang. Occasionally such antennae are supplied with rather long projecting glandular trichomes (*U. longifolia*) (Fig. 14). In *U. Gibbsiae* there is a marked prominence between the stalk and entrance. When these antennae are rather short and thick, and carry a brush of multicellular glandular trichomes curved downward, they characterise a small group of species of which *U. orbiculata* (Fig. 16) and *U. brachiata* (Compton) are examples. In another type, and here we come to that of *U. vulgaris*, and *Biovularia* the antennae, curved up or down, are usually long and slender with a number of delicate trichomatous multicellular extensions, supplemented by delicate combs of similar trichomes arising and spreading outwardly from the cheeks or sides of the entrance, the whole combining to form a sort of funnel-shaped guide to the entrance itself. This arrangement is chiefly characteristic of floating forms (with the exception noted above of *U. tubulata* and of the *U. purpurea* group), and it is this type with which we are most familiar.

And there is finally a type, also not otherwise uniform, in which the tissues around the entrance are expanded into a funnel-shaped approach to the door, the conical structure thus formed being extended above into a sagittally expanded beak, it may be of great length as in *U. albina* (Fig. 15); or into two broad antennae (*U. globulariaefolia*) (Fig. 30) supplemented by a sagittally expanded ramp arising from the surface of the stalk. Whatever the device, the inner surfaces of the conical approach, the under surface of the antennae, and the ramp when present, are clothed with radiating rows or combs of elongated glandular trichomes, directed toward the entrance (Goebel).

In *U. capensis* (Fig. 17), in *U. puberula*, and in *U. Welwitschii* the funnel is less deep and the radiating trichomes are larger than in *U. globulariaefolia*. In the former the combs radiate as above described. In *U. Welwitschii* there is a broad beak (upper rostrum) reaching forward of the entrance, the few glandular trichomes radiating like the spokes of a wheel.

The fact of dimorphism of the traps has been indicated. It occurs in several species, the differences between the two kinds of traps being found in size (*U. caerulea*), shape and amplitudes of development of the appendages, and even in the

presence or absence of some structure (as in *U. Lloydii* to be mentioned later). Thus the contrast between the traps of *U. Warburgi* and *U. rosea* as described by Goebel (1891) is to be found in a single species (*U. albina*) (Lloyd, 1932) (Fig. 15). In the one the beak is very long and straight, relatively; in the other short and downwardly curved (Lloyd, 1932), this being also very much smaller. Somewhat similar
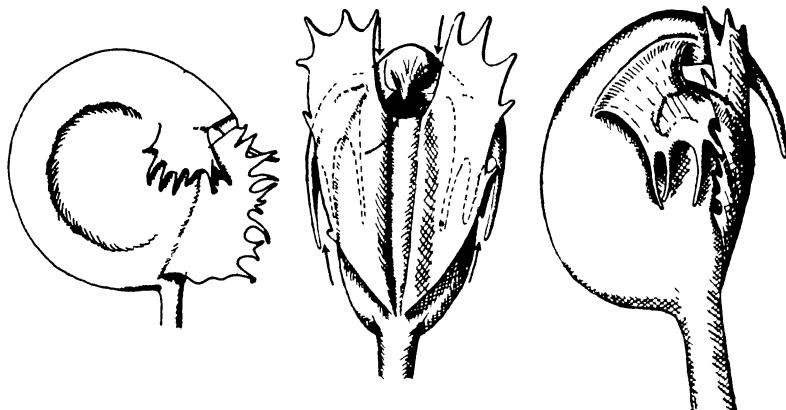
Fig. 12
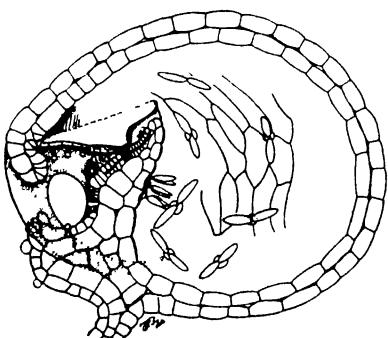
Fig. 13
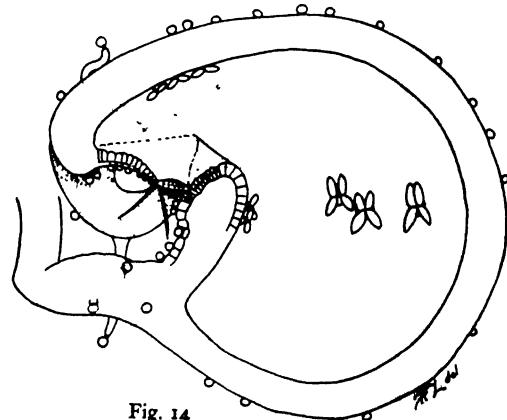
Fig. 14

Fig. 12. *U. monanthos* (left) and *U. Menziesii*, ventral and lateral views.
Fig. 13. *U. caerulea.*
Fig. 14. *U. longifolia.*

differences occur in the group of *U. globulariaefolia* in the two forms of traps, the direction of the stalk is reversed. *U. resupinata* (Fig. 5) has been mentioned above. In *U. Lloydii* (Fig. 18) the two kinds of traps are most strikingly different, one having short sessile trichomes about the entrance, the other much longer projecting ones. The door in one case has a single tripping bristle, in the other none. *Poly-*
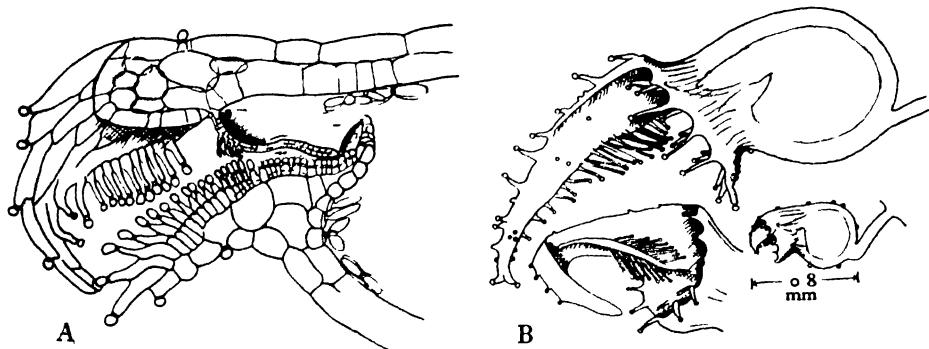
Fig 15
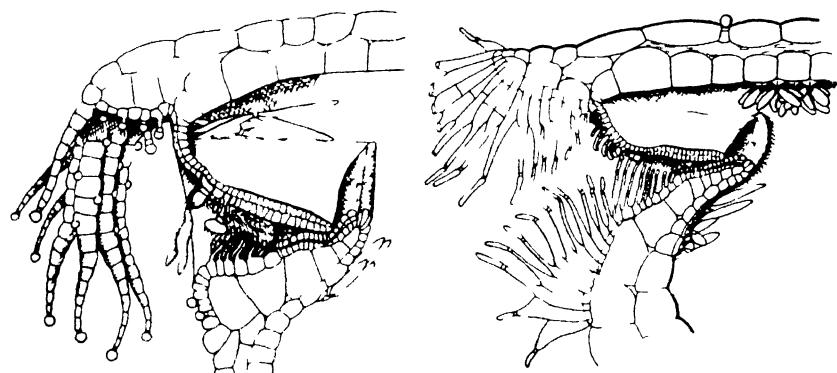
Fig 16
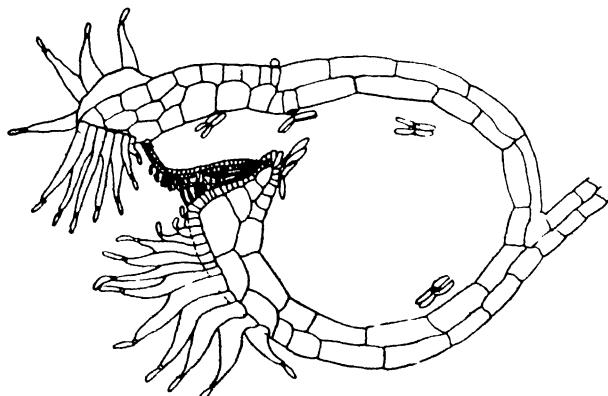
Fig 17

Fig 15 *U albina* A, entrance ; B, two kinds of trap
Fig 16 Left, *U orbiculata*, right, *U puberula*.
Fig 17 *U capensis*

pompholyx also has two kinds of traps, large and small, further characterised by the form of the trichomes clothing the inner end of the threshold (Fig. 18).
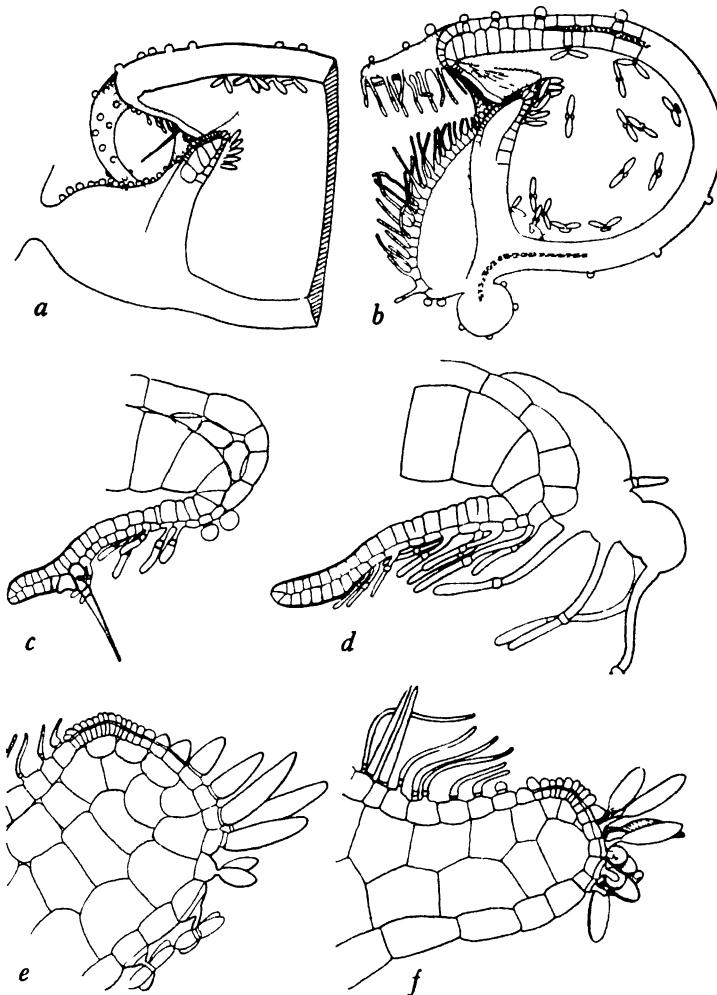
Fig 18

U. Lloydu. a, b, two forms of the trap in sagittal section; *c, d,* doors of *a* and *b*; *e, f,* thresholds of the two forms of trap in *Polypompholyx.*

While there can be no sort of doubt of the occurrence of such dimorphism, it is difficult to offer any explanation for it. The two forms of traps are not regularly distributed on different parts of the plant (except in *U. resupinata*) and seem equally efficient, judging from the amount of caught prey observed. Intermediate forms have not been seen, except again in *U. resupinata.*

One rather curious detail should here be mentioned, namely the occurrence of a few stomata, usually three on each side of the ramp, in such species as *U. modesta*, *U. globulariaefolia*, *U. tridentata* (Merl, 1915). Since the tissues are massive in these species, the walls of the traps being quite thick, and the plants "land forms" though usually submersed in water in the substrate, it is difficult to do more than surmise that they have a function.

VII. THE ENTRANCE MECHANISMS.

(1) STRUCTURE AND BEHAVIOUR.

In previous publications accounts of about 75–80 species of *Utricularia* from all parts of the world have been afforded. The point of departure was found in the structure of the trap of *U. vulgaris* and some of its affinities which had engaged the attention of botanists for fifty years or more. In species other than *U. vulgaris*, the minute structure of the trap (bladder) had had hardly any attention; only very general descriptions, chiefly by Darwin, Cohn, Goebel, Meierhofer, Kamienski, v. Luetzelburg and Schimper had been afforded, but these always stopped short of an adequate account of the entrance mechanisms. This is to be explained by the fact that the view of Cohn and of Darwin, traceable from Treviranus (1848), that the door was merely a passive valve under which the prey pushed their way but which impeded their escape, dominated thought and enquiry. It was not a stimulating point of view, having the disadvantage of not being true. A very different point of view, that the mechanism in question is an irritable-responsive system, though denied by Darwin and only half-heartedly advanced by some others, lately more vigorously by Kruck, had it been successfully supported might have served as a stimulus to enquiry, but it was not. But a third point of view came into position for consideration when, in 1911, Brocher, a French entomologist, observed that before a capture of prey the side walls of the trap were concave, after the capture convex (Figs. 20, 29). He rightly concluded that the capture of the luckless animal was achieved by the sucking in of a volume of water by the outspringing side walls[1]. The retention of the animal was assured by the prompt closure of the door. He further saw that the condition of affairs prior to the capture included a state of "negative pressure" of water in the trap and that this could not be the case unless the door were watertight. This he attributed to the presence of a plug of mucilage; in this he was wrong, though his logic was unassailable. Brocher described the set-up previous to capture as one of "unstable equilibrium," the upsetting of which allowed the side walls to act. He was right in this also, but he further thought that the trap *could act but once*. He made an attempt to explain the stability of the door previous to capture in which there is some little truth, but his observations ceased at this point and there the matter rested until Merl and Czaja, working independently, made similar observations, to the effect that the initial condition of the trap, that is prior to capture, described by Brocher indeed obtains, but that the act of capture can be repeated. This was an

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[1] Brocher noted that on raising a plant out of the water a clicking noise first noticed by Meyen previous to 1848 can be heard. Other observers (Ekambaram, Withycombe and Hegner) also made the same observations independently.

observation of the highest importance, for it led at once to the discovery that the walls of the trap have such physiological properties that they can pump out the water within. The physiology of this process is not understood yet (see Czaja, 1924), but the fact is sufficient for us here. The rate of this expulsion of water by excretion varies, but in two species in which it has been measured it takes 15–30 min. (*U. vulgaris*) (Merl) to about 2 hours (*U. purpurea*) (Lloyd). This means that these periods
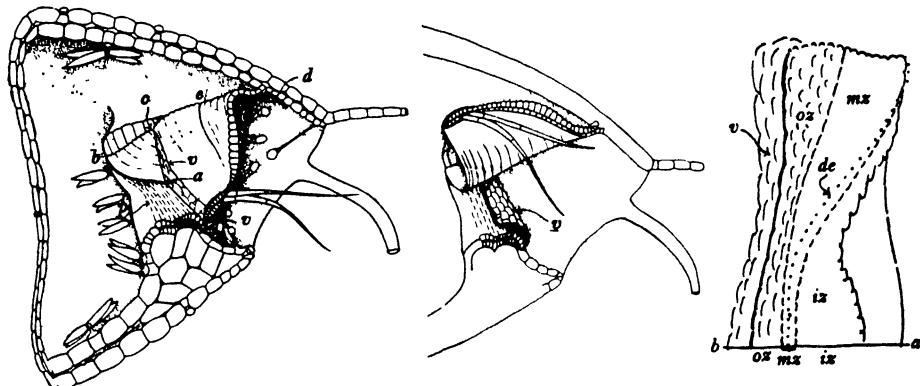
Fig. 19
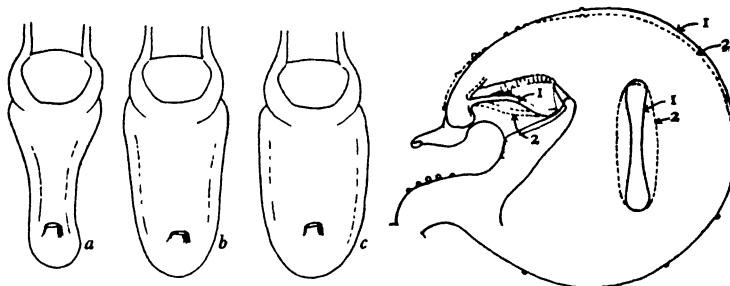
Fig. 20

Fig. 19. *U. gibba*, with the door in closed and open postures, and the threshold as viewed from above. *b*, *e*, *d*, attachment of outer hinge to wall; *v*, velum; *p*, pavement epithelium; *a*, *b*, *c*, area of door applied to the threshold; *iz*, *mz*, *oz*, inner, middle and outer zones of pavement epithelium; *a*, *b*, sagittal line.

Fig. 20. Left, *U. intermedia*, dorsal views of a trap set, sprung and after puncture (*c*). Right, *U. caerulea*, diagram showing change in form of trap in lateral view set (1) and relaxed (2) posture.

are required for sufficient exhaustion for the trap to be set in working order again; that is, to produce sufficient convexity of the side walls to cause an inflow of water if and when the trap is actuated. Obviously there is a concurrent action of the door, for after actuation it must resume its position exactly if it is to repeat its effective exclusion of outer water during the period of exhaustion, but how this is achieved remained unexplained. That the four bristles inserted on the middle line of the door

near its lower free margin constitute a sort of tripping mechanism leading to the upsetting of the "unstable equilibrium" was recognised, but no precise explanation of its method of action was forthcoming. Nor was any explanation given of how watertightness is brought about.

At this point Withycombe (1925) saw clearly that the logic of the situation called for a mechanism capable of repeated action which between whiles, should be watertight. He concluded from the study of paraffin sections that the stability of the door is assured by the resting of the free edge against the outer edge of the pavement epithelium in a sort of groove, and that, arising from nearby epithelium of the entrance and in front of the door edge, there is a strand of mucilage which makes the door edge watertight, as a bolster prevents draughts from creeping beneath a door. In this view he departed from the generally accepted and first enunciated view, that the door edge, like that of a simple valve, lies inwardly bent with its outer surface applied to the inner edge or to the top of the threshold. But though his logic led him to a correct view of the matter, he failed on both counts to determine the objective facts, and it reflects the difficulties of the problem to mention that later students still adhered to the earlier view or a close equivalent (Czaja, Kruck). Withycombe, further, was one of those who accepted the idea, without any real enquiry into the matter, that the system is a stimulus receiving and thereto reacting one, to which even Merl gave an equivocal adherence.

At this point my own curiosity was awakened. Feeling that mucilage was a poor kind of mechanical hindrance to the inflow of water under pressure between the door and the threshold, I searched for and found the mechanism in a definitely organised membrane consisting of the mutually adherent cuticles of the "pavement epithelium," as Goebel called it, which, however, merely balloon away from the outer zone thereof (Fig. 21). The whole, therefore, remains attached to that zone of pavement epithelium just in front of the door edge. This membrane, impervious to water, forms a second valve overlapping the first, the door, in such manner as to quite close the chink between the latter and the threshold, so far as there is a chink. The very delicate and diaphanous quality of this membrane, which I call the velum, had caused it to remain unobserved hitherto, except that Giesenhagen had drawn it in a semi-diagrammatic sketch made for a publication of Goebel (1891). As it escaped comment in that and later publications, it is fair to infer that it was not seen by the eye of the mind, and a feeling of personal loyalty to my great teacher prompts me to add that he generously acknowledged the oversight; as a matter of fact he had been less concerned with such matters than others. It is fairly certain also that Withycombe too saw the velum but, as in his preparations it would have remained unstained, being cuticle, it must have looked, as I know from my own experience, like a transparent unorganised mass. To complete the record it should be added that R. E. Fry, while a student at Cambridge many years ago (*ca.* 1891), prepared a thesis, never published, illustrated with excellent pencil and colour drawings, in which he also showed the velum, but merely as an unexplained anatomical detail. Such observations, though undermining personal vanity, yet supply substance to the contention.
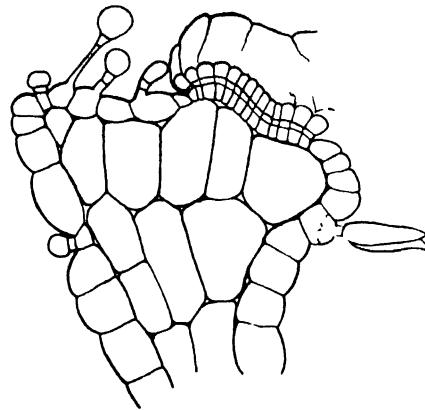
Fig. 21
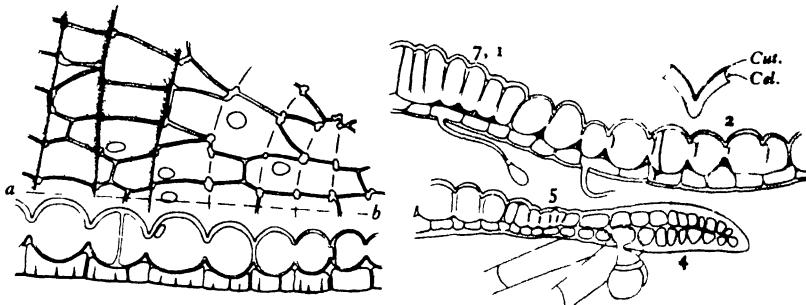
Fig. 22
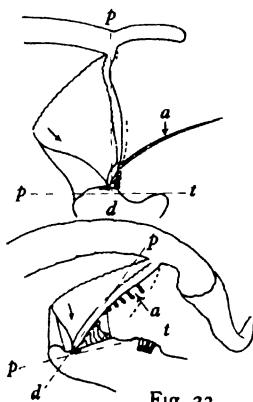
Fig. 23
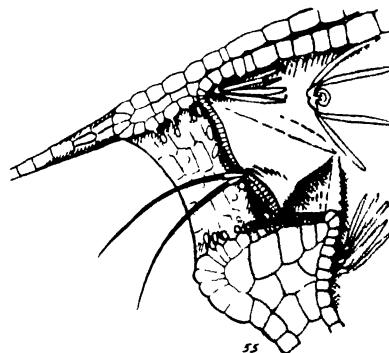
Fig. 24

Fig. 21. *U. gibba.* Diagram of the sagittal section of the threshold indicating the origin of the velum.

Fig. 22. Diagrams of the cellular structure of the door (*U. intermedia*). *Cut.* cuticle; *cel.* cellulose wall; 7, outer hinge; 1–2 middle area; 5, central hinge; 4, middle piece.

Fig. 23. Diagrams of the two types of entrance mechanisms. Above, with wide door-threshold angle; below, narrow angle. *p, d,* plane of door; *p, t,* plane of threshold; *a,* approximate points of activating thrust; pointers, direction of thrust of lateral hinge areas on the middle piece.

Fig. 24. *U. neottioides.*

The discovery of the velum naturally led one to a closer examination of other details. It was found that the door edge and not its outer face rested on the threshold, and, further, that there is a definite line or position of its application. This is always against the middle zone of the threshold, which has its own anatomical characters, namely, the very tight packing of the outer or capital cells of the glands which constitute the pavement epithelium. Their cuticle gone, they are soft and yielding and receive the door edge as on a soft thin cushion, whose outer zone is more or less uptilted for the better and more stable reception of the door edge. Actuation of the trap as a whole follows only on release of the door edge by a disturbance of the bristles, when these are present, or by some mechanically equivalent action.

It is important to note, however, that it is only the middle reach of the door edge that is placed as just described. Since the ends of the door edge are attached to the inner angles of the threshold, while the middle reach of the door edge lies in front of its middle zone, the lateral reaches must traverse obliquely the corresponding areas of the threshold. Here, therefore, the outer surface of the door does lie against the threshold, as described in the now classical literature of the subject[1], and here, therefore, is the greater need for stoppage against inflow of water; and I have shown experimentally (1932*b*) that damage to the velum in these regions puts the trap entirely out of action. The velum here is indeed wider and deeper, as it must be to reach over and fully plug the rather wide fissure between the incurving fold of the door and the threshold, forming a re-entrant angle offering no great, if any, resistance to water under greater outer pressure. In the lateral reaches of the threshold also the middle zone is wide, occupying the full width from the posterior edge of the velum (outer) zone, and is, moreover, tilted inwardly so as to produce a slightly conical receptive surface into which the door is cramped.

Since the door edge traverses the threshold obliquely (Fig. 19), the latter having a considerable width while the door edge is thin, it follows that the latter must be longer than the threshold. This very condition prevents the door from moving inwardly under water pressure, contributing to the stability of the system, especially as the door edge digs into the pavement epithelium. It further follows that, if the door swings inwardly when the outer water pressure is allowed to act, it cannot do so without suffering distortion. What happens is that the door edge folds longitudinally, *i.e.* along its sagittal axis, allowing the edge to slip over the threshold, its mucilaginous secretion facilitating the movement. The following reversal of curvatures of a roughly quarter-spherical thin and pliable yielding membrane, the door, under pressure of a column of water, can be more briefly and easily represented by diagram (Fig. 19).

As soon as the pressure of the column of water relaxes sufficiently, the door swings back against the tide which, when at its ebb, allows the door edge to fit itself back into place between the middle zone of the threshold and the velum[2]. The

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[1] Being free of cuticle, the capital cells of the pavement epithelium are yielding and remain undamaged by distortion. Darwin (1875) observed this to be true (*ret. par.*) of the quadrifids.

[2] The walls are thus prevented from complete expansion, which can occur only if some object catches under the door edge, or if the velum has been damaged (Lloyd), or if the walls have been punctured (Merl, Czaja) (Fig. 20).

excretion of water by and through the walls of the trap now continues until a more pronounced condition of unstable equilibrium is re-established.

The swiftness of this action deserves comment. It will be recalled that it sur-prised Darwin, who remarked: "On three occasions minute particles of blue glass... were placed on valves while under water; and on trying to move them with a needle, they disappeared so suddenly that, not seeing what had happened, I thought that I had flirted them off; but on examining the bladders I found them safely enclosed."

By making use of motion photomicrography, it was found that the entire action falls within the limits of two following exposures at normal speed (16 frames per second), so that, when projected, the moving picture merely shows the beginning and the end of the action. When the camera is speeded up to 160 frames per second, the whole action falls within the sequence of five frames, the opening phase falling between two frames and the slower closing phase occupying the rest of the time. During this very brief passage of time the door moves through a considerable arc of opening and returns to its former exact position (Fig. 19). The action is purely a mechanical one, assuming of course the physical properties of the door and threshold as living organs having turgidity, flexibility and the rest. Thus the door entrance mechanism could act again at once if only the "negative pressure" of the interior of the trap could be rapidly enough renewed, which is not normally the case. We may, however, prove the point experimentally, and I have in this manner shown that the door action can be made to repeat several times in a minute so long as the manipula-tion (pressure on the side walls) is delicate enough to avoid damage to the trap[1]. Kruck's contention that 15 min. are required for a restitution of irritability is with-out substance; nor is she right in her description of the change in door posture during that 15 min., as has been shown by comparison of the silhouette of the trap before and after action (Lloyd, 1932 b). Differences, small in amount, there are between the before-action and after-action contours (Fig. 20), but such differences can be accounted for by referring them to the more or less relaxed condition of the trap as a whole, depending on the differences of water pressure before and after action.

It remains to account for the physical characters of the door, particularly its flexibility rather than its elasticity, a word not well chosen whenever it has been used (Fig. 22). This property has scarcely ever remained unremarked (Darwin, 1875), nor has its explanation escaped earlier observers, namely that the inner surface of the door is circularly corrugated about a centre at a point marked by the insertion of the tripping bristles. Darwin, Meierhofer, Goebel and others noted this. There has been less unanimity on the exact anatomical details.

Roughly speaking, the door consists of two courses of cells; an outer usually thin, the inner thicker. In some species these relations are reversed locally, to be explained by the particular mechanical conditions displayed by different parts of the door. The cells of the outer course are more or less zigzag in outline with thick props[2] (Figs. 4,

[1] Darwin (1875) used this method to determine that air could escape from the "bladder" only through the chink between the door edge and the "collar" (threshold); and Ekambaram obtained repeated action (1916).

[2] That is, more massive rod-like portions of the anticlinal walls.

22) at the angles of the walls. It is relatively rigid, but is at the same time flexible without danger of collapse of the constituent cells (because of their reinforcing props). It affords a sort of fulcrum for the inner course of cells, giving sufficiently for the latter to enjoy freedom of movement. The cells of the inner course are elongated in the direction of the radii centred on the very thin area of the door (the middle hinge), placed with its centre just above the insertion of the tripping bristles. The outer walls of these cells are strongly corrugated transversely to their long (radial) axes and are continued across from cell to cell without respect to the positions of their ends, which are various. The corrugations therefore are continuous and very regularly circular, as above stated. As a corrugation passes over a cell it is somewhat bowed or arched, so that viewed obliquely one does not get the vision of a simple fold or corrugation but of one repeatedly curved as it passes over each cell. The re-entrant angle of the transverse fold has much the optical effect of a cell wall, that is a transverse wall of the cell beneath, or of a thickening as a bar, and this has misled some observers to describe the inner course of cells as isodiametric. Each transverse arch of a corrugation as it crosses the cell beneath is supported by a strong pillar or prop in the anticlinal walls at the point of crossing, producing in effect a bow-shaped, reinforcing support which has been described as a ring thickening.

Where, as along the side areas of the door, along its free edge, and in the outer hinge area, there are great folding strains to be met when the door is widely opened, both the cells of the inner as well as the outer course are elongated radially, and those constituting the thickened door edge (more in the middle reach than in the later reaches) are very minute and are richly supported with props. The concentric corrugations in the outer hinge are much narrower and more numerous, and have generally escaped observation.

The total effect of this highly specialised anatomy is to produce a door or valve of great flexibility and resilience, and, by way of analogy, it may be compared with the slightly opened bellows of a concertina stabilised on one side with a sheet of india rubber attached to the edges of the bellows. Manipulation of the business ends of the concertina in such an instrument would be sadly hampered as far as the normal purpose of the instrument is concerned, but it serves to illustrate the mechanical properties of the door. The analogy might be pushed further but to no greater profit. The door is, however, a much more adaptable affair, since the walls of the cells are tough but yielding and the cell walls, stiffened sufficiently by the self-maintained turgor, procure an organ capable of flexure in all directions, while the pitiably inefficient bellows of our concertina can be flexed in only one plane. The old-fashioned hexagonal instrument approaches the condition somewhat more closely.

The doors of the various species of *Utricularia* are by no means alike in shape, (Figs. 27–34) nor do they work all quite in the same way, but these differences will be made clear below. It is sufficient here to say that they display a remarkable uniformity as regards their anatomical structure, aside from the matter of tripping bristles, glandular trichomes, etc.

We return here to the structure of the threshold, which is, as has always been

recognised, the most massive and rigid part of the trap (*e.g.* Darwin) (Fig. 19). The threshold, with whose upper surface the door comes in contact, is a thick weal of tissue ("collar," Darwin) forming an arch in the lower half of the entrance and remaining constant in shape, while the walls elsewhere show changes with differences of water exhaustion.

There are two different forms of threshold—narrow transversely and broad[1] (Figs. 4, 19). The former, viewed along the axis of the trap or its entrance, is nearly semicircular and is relatively large, as in *U. vulgaris*; the latter is narrow or more constricted, and embraces perhaps two-fifths of the circle in its arch. This difference is correlated with the shape and position of the door, the plane of which in, for example, *U. vulgaris* or *Biovularia*, passing through a sagittal element, lies nearly at right angles with that of the general surface of the threshold; while in the broad type (*e.g. U. capensis*, *U. monanthos*) this angle is much smaller, the door slanting inwardly (Fig. 23). For this apparently disadvantageous mechanical arrangement, in which a large re-entrant angle might be expected to offer little opposition to the influx of water, there are compensations in the moulding of the pavement epithelium, especially toward its inner margin, in a much more ample velum, and in the posture of the door edge itself, which is cramped into the conical arch of the threshold, contributing, by its very firm application to the threshold surface, to the end of excluding water. Further details of this also must be deferred. In all cases the surface of the threshold is always more or less slanted so as to face outward, so that, as the outer water pushes on the door surface, its effect is to cramp the door into the arch of the threshold. This contributes in all cases to the greater stability of the unstable equilibrium. The rigidity of the threshold bolster, as it has also been called, is evidently an important contribution to delicate equilibration.

In conclusion of the preceding, it must be evident that the entrance mechanisms of the traps of *Utricularia* are far more complicated and more delicate in their adjustments than has heretofore been thought. The descriptions which have prevailed and from which text-book illustrations have been derived have been wholly inadequate in enabling the reader and student to form a true conception of the matter. One would be ill-advised to think that we know all about it yet, but the evidence compels the opinion that the mechanism in question is not an irritable one but purely mechanical in its action, and that its form and anatomy converge in a complicated and delicate fashion to its purpose (using this word in the sense that Sachs used it).

(2) COMPARATIVE TREATMENT.

While it is possible to arrange a series of forms as respects the entrance mechanism in terms of relative complexity (more, however, as a matter of convenience in description than other), any such series which may be arranged will be found to run athwart any similarly arranged series with respect to the general characters of the appendages, etc., as above described; for while in some cases these two sets of anatomical facts may be rather closely correlated (as in the *U. dichotoma* group)

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[1] As measured along the sagittal line of the trap.

there are other groups in which, the external features being almost identical, the entrance mechanism is wholly different (*e.g.* *U. Dusenii* or *U. longifolia* and *U. caerulea*).

In the older literature there is an entire lack of anything like accurate descriptions of the door and threshold and their true functional relations. Even when the structural features of the parts concerned have been treated with some detail, as in the case of *U. vulgaris* (Cohn, Meierhofer, etc.), the significance of the mechanism in its entirety has been missed. Other species have received some consideration chiefly at the hands of Goebel, v. Luetzelburg and Merl, but their observations, not having been impelled by some motive to exhaustive examination of details, invariably fall short of accuracy. The point of view which permits this criticism is set forth in a previous part of the present paper. In the following it will be necessary to describe the door, its parts and their function, its posture with relation to the threshold, and the structure of the latter with reference to the emplacement of the door edge and of its velum.

It will be fitting to recall that there are two sorts of trap (Fig. 23) which, while conforming in general physical principles, differ in the posture of the door relative to the threshold: (*a*) that in which the plane of the door (*i.e.* the plane tangent to the median element of the door surface along its middle line) stands at a large angle (*ca.* 90°) with the general level of the threshold surface (*e.g.* *U. vulgaris*, above described); and (*b*) that in which this angle is a small one—less than *ca.* 30°. The mechanical difference in action related to these differences in posture will be elucidated as we proceed. The diagrams herewith will assist in visualisation of the two sorts.

(a) Traps with a wide door-threshold angle.

With tripping mechanism of bristles. The type of door mechanism displayed by *U. vulgaris* has already been described sufficiently. Completely conforming with it are such species as *U. gibba* (Fig. 19), *U. intermedia*, *U. emarginata*, *U. inflata*, *U. stellaris*, *U. exoleta*, *U. neottioides* (Fig. 24)—freely floating or anchored submersed forms.

Very similar is a series of forms which are anchored and "terrestrial" in habit. The more striking differences are to be seen in the more massive character of the middle piece of the door, and in some instances in the position of the threshold surface. That the mechanism is in all respects similar in behaviour to that of *U. vulgaris* has been determined by examining living material of *U. reniformis*, *U. longifolia* and *U. Dusenii*, which with *U. Humboldtii*, *U. nelumbiifolia*, *U. subulata*[1], *U. resupinata*, and doubtless others will be seen to include the most showy and largest representatives of the genus, which for many years have been in cultivation and as much appreciated for their size and delicacy of form and colour as orchids. In all cases the

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[1] *U. subulata* is one of only three species of this lot which are said to be found also in the Old World (tropical Africa). The African plant is, however, distinct; it will be sufficient to mention here that the arms of the quadrifids in the latter are very long, slender and sinuous, and the antennae longer, while in the American plant the arms are short and thick and the antennae shorter.

tripping mechanism consists of normally four pointed bristling trichomes affixed near the middle line of the door at the upper edge of the middle piece. In all the forms represented by the species mentioned in this paragraph, the middle piece is deeper and thicker than in the floaters.

The type *Biovularia* (Fig. 27). The trap of this plant which is also freely floating, stands between *U. vulgaris* and *U. purpurea*. In the general form and proportions, as also anatomy, of the door it resembles *U. purpurea*, in the tripping bristles *U. vulgaris*, except that there are normally six of these placed in a transverse row, inserted as in *U. vulgaris*, as there is no knob. The threshold appears to be as in *U. vulgaris* (Lloyd, 1934).

Tripping mechanism of glandular hairs. The type *U. purpurea* (Fig. 25). Certain details of structure of this type were known and described by Goebel and v. Luetzelburg. The ventral border of the trap is straight while the dorsal border is curved over in such a manner that the threshold shows little or no torsion, its pavement epithelium lying parallel to the ventral profile. In the middle of the door, projecting from its outer surface, there is a knobby mass of tissue which bears a number of radiating glandular trichomes with a long basal cell, short mid-cell, and a globular or spindle-shaped capital cell (according to species) with a much expanded spherical cuticle. Sometimes a degree of dimorphism appears, as in *U. purpurea*, where there are two kinds of these trichomes distinguishable in size and shape. They constitute a tripping mechanism of great delicacy of action. The knob is surrounded by a thinner portion of the door, constituting a middle hinge which permits its rotation in various directions, this in turn resulting from the disturbance of the trichomes by the jostling of a small animal. This disturbance may be in any direction, as shown experimentally. The rotation of the knob in any direction equally affects the position of the broad middle piece, which is thereby released from its stable contact with the threshold and so permits it to swing inwardly in front of the incoming water column. The outer hinge which permits this swing is sigmoid in form, the relative thickness of the two courses of cells being reversed as the knob is approached. The stable position of the door edge is assured by the swollen cuticles of the inner zone of the pavement epithelium, which, contrary to their behaviour in other types, remain entire. The velum consists of the much-ballooned cuticles of the outer zone, and their efficacy as a velum is contributed to by a flange or beading on the forward aspect of the door edge. Conforming to this description are *U. cucullata*, *U. elephas* and *U. myriocista*, distinguishable by the presence of an upturned lower rostrum and other less noticeable characters. They are wholly confined to the New World. While they are regarded as floaters, this may not be taken as wholly true, as in *U. purpurea* there are very slender stolons embedded in the mud in rather deep water which serve for anchorage.

The type *U. longeciliata* (Fig. 26). The general features are much as in *U. subulata*, etc., except that there is, as Merl showed, only one glandular trichome present to function as a tripping part. How efficient this is has not been ascertained, as the plant has never been studied in the living condition. The glandular tripping trichome assimilates the plant to *U. purpurea*.
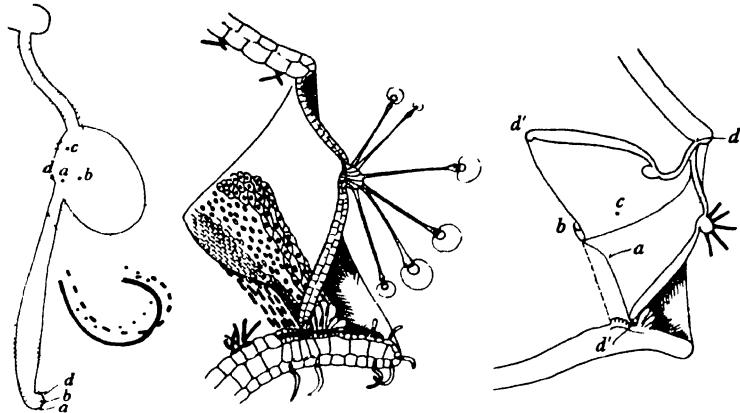
Fig. 25
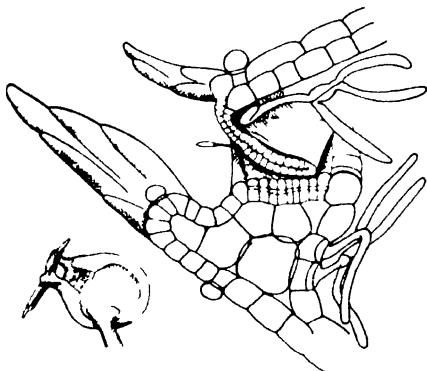
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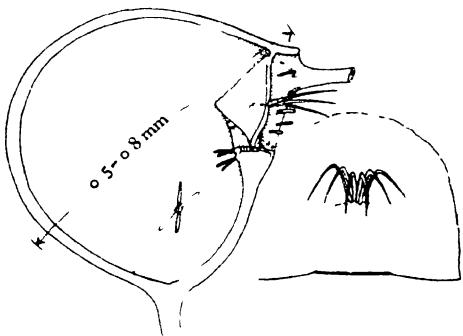
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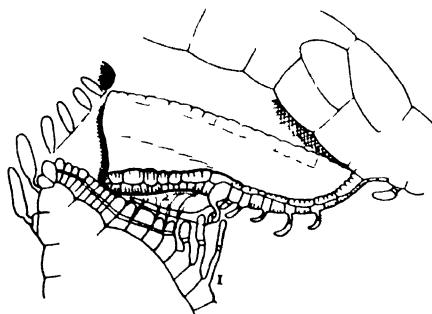
Fig. 28

Fig. 25. *U. purpurea.* Entrance mechanism showing structure (middle) and with door closed and open; (left) the door in set (*a*) and released (*b, d*) postures.
Fig. 26. *U. longeciliata.*
Fig. 27. *Biovularia.*
Fig. 28. *U. Welwitschii.*

The type *U. nana* (Fig. 33). With a general likeness to the above, the door has always only two tripping bristles with slightly swollen basal cells.

The type *U. Lloydii* Merl (Fig. 18). This small Brazilian plant has, as already shown, two forms of traps, distinguishable, among other features, by the presence in one of them of a single tripping bristle. This is stiff and tapering, with a peculiarly expanded basal cell supported on one side by a buttress cell, the whole forming a stiff but flexible hinge.

Tripping mechanism of sessile hairs. Here we must place a small group of species, including *U. globulariaefolia* (Fig. 30), *U. amethystina*, etc. These, while very far from a close resemblance to *U. vulgaris*, etc., in general form of the trap, owing to the elaborate character of the approach to the entrance and in the absence of a tripping mechanism (unless we may regard a small group of sessile trichomes as such), nevertheless accord with this form in the narrow threshold and the position of the door in relation thereto. The velum is not an ample one, but the long and numerous trichomes of the entrance with glandular capitals lean against the door on all sides, exposing only a central spot against which the animal about to be entrapped must butt to effect indenting. The door itself is rather thick and clumsy looking but is, in spite of appearance, very flexible. Contributing to this is the highly complicated system of corrugations of the inner surface, allowing much freedom of bending in every direction. In some species the door edge is supplied with a ridged flange which appears to dig into the pavement epithelium the more to assure fixity of door position when the trap is set. A further feature contributing to the total activity of the trap is to be seen in its thick walls, which would the easier overcome any increased resistance of the door to the inflowing column of water owing to its apparently greater massiveness.

(b) Traps with a narrow door-threshold angle.

There are two distinct categories under this head, namely traps in which the door engages the threshold by pressure of its free edge against the concave more or less outwardly fitted threshold, and those in which the door engages the threshold by means of its outer surface, the door edge being bent over the threshold where itself is reciprocally bent (a purely Australasian type). These two categories are presented separately.

Without tripping bristles. The type *U. cornuta* (Fig. 4). As observed above, the trap is devoid of appendages. In the entire absence of guides thought to lead the prey to the entrance (a view resting on the apparent significance of the antennae in *U. vulgaris*), there is, however, a structure, observed by Schimper, which may be a lure, consisting in a small oval patch of glandular cells just below the entrance (Figs. 4, 29). The wall of the approach to the door or vestibule is also clothed with clavate glandular trichomes which may contribute to the lure.

In strong contrast to *U. vulgaris*, the door of *U. cornuta* has no tripping bristles and the only trichomes present are small sessile ones with sausage-shaped capitals. There is, however, a tripping mechanism, as will be seen. The door is hung from the under side of the projecting fold of the wall, or beak; indeed the underside of the
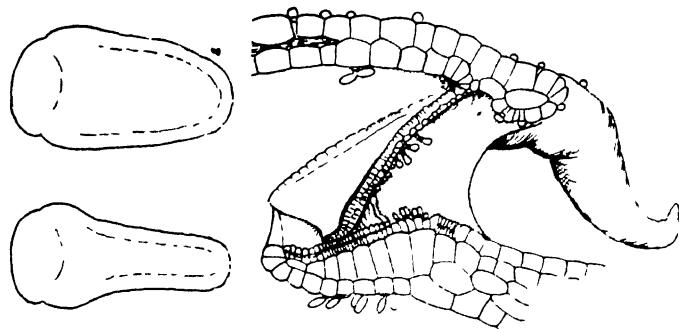
Fig. 29
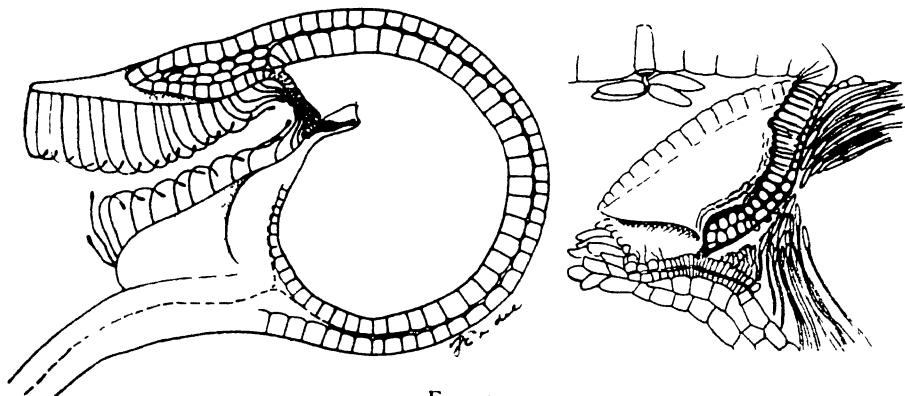
Fig. 30
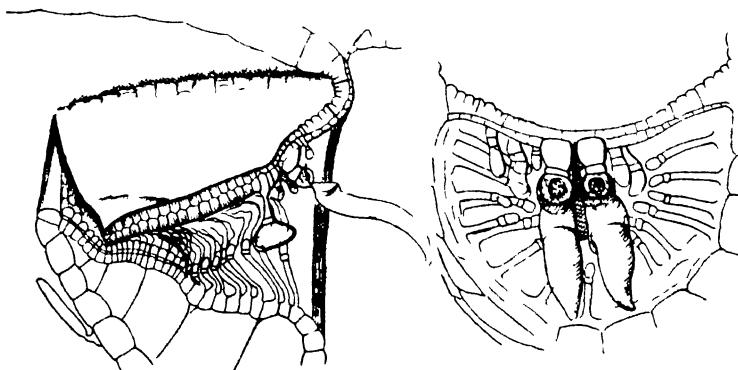
Fig. 31

Fig. 29. *U. caerulea.* Left, a trap set and after actuation.
Fig. 30. *U. globulariaefolia.*
Fig. 31. *U. orbiculata·*

whole beak contributes to the door mechanism, but histologically the door can be distinguished from the beak. If we examine the entrance mechanism of a trap in a completely relaxed condition, as must be the case if a trap be sagittally split in order to display the structures, the door (including the under side of the beak) will be seen to be convex, sweeping downward and backward to meet the threshold with a small angle of contact (Schimper). The latter is anteriorly-posteriorly broad, and relatively narrow and strongly arched downwardly. The forward half of the pavement epithelium supplies a broad velum consisting of the ballooned cuticle of its cells, while the inner half, being compact, is meet for the firm reception of the door edge when the trap is in the set condition, at which time the posture of the door is far different from that above described and as represented by Schimper[1] (1882) (Fig. 4). In this condition the side walls are, of course, concave, indicating that the "negative pressure" now obtains. The water on the outside of the trap now exerts a greater pressure on the door than that within. Due to this and due partly to the strain set up by the walls on being withdrawn into the concave position, the beak now becomes somewhat more sharply bent and the door concave. Its free edge being now applied firmly to the middle zone of the threshold, owing to the cramping of the door into its somewhat funnel-shaped sides, and the now greater angle between door edge and threshold being stopped by the velum, the whole is watertight and stable against the outer water pressure. The door may now be tripped by pressure against the outer surface[2], which must be sufficient to indent it to some extent, when the resistance of the curved surface is thrown out of commission and the inpressing water can cause the extension of the flexure backwards to the door edge. The door can now sweep upward in front of the inrushing water, to return to its original relaxed position on the slacking of the tide (Lloyd, 1933 c).

It will be suspected that this arrangement is mechanically not as delicate of action as that in *U. vulgaris*; nor is it. I have repeatedly activated the traps of this and another form which agrees mechanically (*e.g. U. caerulea*) and there can be no doubt that, while the affair is sensitive enough for the purpose of catching prey, they are not caught on any such slight contact as suffices in traps with tripping bristles; they require, as in *U. caerulea*, to press with some urge against the door surface. Incidentally it can here be pointed out that no response can be had merely by touching the door surface anywhere, even when repeated as often as one cares, so that there can be no doubt that here we are not dealing with an irritable mechanism, as maintained by Kruck in the case of *U. vulgaris* but to which an answer has been given (Lloyd, 1932 b). Since this type of mechanism is scarcely to be found in any but the land forms, mechanically favourable conditions for acting on the door are, it may be surmised, often favoured by immediate contact of the substratum, aiding minute animals by affording them a foothold to exert pressure which they could not if surrounded only by water. This, however, is by no means necessary.

The method by which the facts relating to the changes in the posture of the door

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[1] My own earlier figure (1931, Fig. 11), equally fails to show more than the relaxed position of the door, and is now superseded by Fig. 3, 1933 c (see Fig. 4 above).

[2] The mechanically most sensitive spot is just above the velum.

were determined should be mentioned. This is to photograph the trap in the set and relaxed condition, so as to obtain a silhouette of the door and threshold in each case. The opacity of the trap in the entrance region precludes the obtaining of much detail, nor does infra-red help us in this regard; but sufficient can be recorded on the plate to enable us to see the differences in posture (Lloyd, 1932*b*) of the door with a high degree of exactitude. These differences can be visualised the better by optical projection of one picture over the other, and the results are seen in Figs. 4 and 20.

Disregarding differences in anatomical detail, which in some cases are quite great, it can be shown that the mechanical conditions for the activation of the door described for *U. cornuta* are repeated in a series of forms, and the facts will now be briefly summarised, the species mentioned being regarded as types of groups inclusive of a smaller or greater number of species which it is not necessary to itemise.

The type *U. caerulea* (Figs. 20, 29). So very similar is this to *U. cornuta* that it is necessary only to point out (*a*) the presence of a graded clothing of club-shaped (above) to sessile (below) trichomes on the outer door face toward the lower edge, where there is a group of sessile trichomes with transversely expanded capital cells; and (*b*) that the middle piece of the door is slightly thinner along the free edge. The effect of this is to allow this thinner band of tissue to be appressed against the threshold when the door, on exhaustion of the trap, takes its resulting sagittally concave posture. This concavity is as in *U. cornuta*, owing to the flattening of the transverse curvatures, putting the whole into a mechanically sensitive condition.

The type *U. puberula* (Fig. 16). This type accords with *U. caerulea* in having no tripping bristles, but we may regard a very few, rather large, globose, sessile capitals of glandular cells to be such. The upper moiety of the door is here strongly convex (outwardly) and is thin. It bears a dense group of clavate trichomes covering most of the middle front surface, and at its lower limit the large globose trichomes. The middle piece is deep and thick, with a thick outer wall, and lies in contact with an ample velum similar to that of *U. caerulea*. Activation of the door follows the indenting of its convex upper portion. It is not without interest that this species is (probably) the only New World species with a form of door common to numbers of Old World forms, such as *U. capensis* described below. The approach to the entrance is similarly peculiar in this. The threshold is of the *U. caerulea* kind.

A group of asiatic species (*U. albina*, *U. rosea*, *U. Warburgii*) (Fig. 15), otherwise of very peculiar forms as regards the appendages as described by Goebel, accord with *U. puberula* in all respects save that the large globose trichomes are absent.

The type *U. lateriflora* (Fig. 8). This Australasian species, together with a Ceylonese species (Simpson 9482) which may be identical with *U. calliphysa* Stapf from British North Borneo, have the same form of door as the above. The bacilliform trichomes, however, are few and closely packed and centrally placed on the middle of the upper moiety of the door to form a tripping mechanism analogous to the globose trichomes of *puberula*. The traps are very minute (0·3–0·5 mm.).

The type *U. Welwitschii* (Fig. 28). Compared with *U. caerulea* there are here slight differences in the curvatures of the door, but these do not affect the matter

of general postures. The upper half of the door outer surface bears a group of clavate trichomes, while at its lower limit is placed a single large and curiously shaped trichome (kriss trichome) which may be the tripping mechanism. *U. capensis* (Fig. 1) has an almost identical arrangement, the kriss trichome being less curved.

The type *U. orbiculata* (Figs. 16, 31). This represents a group of species of very uniform habit favouring wet moss or tree trunks, ledges, etc., provided with minute and numerous tubers in some cases and circular expanded leaves.

As regards the door, excepting for its general form, which is that of the above except for slight differences in curvatures, these species are very peculiar in having as a tripping mechanism a group of three trichomes placed at the angles of a triangle with its apex at the middle and upper limit of the middle piece and its base in the contiguous lower portion of the upper moiety of the door. The trichome at the apex is a large (mason's) mallet-shaped affair, the capital cell much expanded and somewhat oblique, and in position and function can be compared with the kriss trichome of *U. capensis*. The other two trichomes are sessile, and when young have an enlarged oblique base set in an incurving dent of the door, a disc-shaped mid-cell and a large globular capital. On maturity this is further enlarged by the formation of a considerable mass of stiff (in water indispersible) mucilage or gell. The cuticle finally bursts in a definitely regular fashion and the gell expands into a long horn-shaped curved mass which protrudes in front of the entrance of the trap, there being of course a pair of these. The three trichomes are so placed that approach to the entrance is impossible without pushing against them, and they may be considered collectively as a tripping mechanism. The broad bases of the horn trichomes, being set in a thin part of the upper hinge, may be seen to be in a good position for distorting the door when disturbed. It is doubtful if *U. multicaulis* Oliv. (Fig. 10) is related to *U. orbiculata*, as thought by Oliver (1858), though it is undoubtedly of the broad threshold sort.

This type is further peculiar in having a secondary adjuvant velum consisting of the cuticles of stalked glandular trichomes forming a thick covering of the sides of the entrance leading directly to the threshold; the latter is supplied with the primary velum as usual. As we shall see below, in many Australasian species this secondary velum is found still more developed into a circular velum.

With tripping bristles. The type *U. Kirkii* (Fig. 32), the only species known to have the following structure. The door has a relatively extensive upper part, uniformly thin and strongly convex, the relative thickness of the two cell courses being reversed on approaching the middle piece, making a middle hinge. On its surface are a number of short clavate trichomes, and at its lower limit two strong curved bristles which extend downward and forward. The middle piece is very thin along its middle line, but on each side of this there is an oblong swelling, giving the necessary stiffness to the whole. At the same time, this arrangement permits the middle piece to bend readily along its mid-line, giving to the whole a great delicacy of response. Toward the free edge the middle piece grows thicker and the edge itself is broadly blunt. In the set position the middle piece is tilted up so as to form a rather wide angle with the threshold surface. In this adjustment the middle hinge is made use of.

Although this type is placed in this position of classification, it can readily be seen that in the possession of the two tripping bristles it resembles the forms with such a mechanism, namely the *U. vulgaris* type, in particular *U. nana*. But the door in general form is distinctly comparable with that of *U. puberula*, etc., though in the presence of the two swellings of the middle piece (derived by the overgrowth of cells of its inner course) it is unique.

(c) Traps with door overlying a transverse ridge or bend of the threshold.

These are all, so far as known, Australasian, and are quite uniform in general structure. There have been studied *U. monanthos* (in the living condition), *U. dichotoma*, *U. Menziesii* (Fig. 34), *U. violacea*, and *U. Hookeri*, all "terrestrial," that is, anchored in shallow water; and *U. tubulata* (Fig. 35), a floating form from Queensland. The two species of the genus *Polypompholyx* conform also to the type.

Their peculiarities of structure are so great that it is necessary to particularise more fully the door and threshold. *U. monanthos* is chosen as typical of the group. The door is of an extremely narrow form, the free edge being correspondingly short, resulting in a strong lateral thrust of the outer hinge on the middle piece. It is uniform in thickness, but the middle piece is much strengthened by a thickened outer wall, imparting, with the very small cells, a distinctly cartilaginous texture. The upper moiety of the door is very flexible and bears a number of low glands in the upper part. In the relaxed condition, this portion of the door bulges forward, strongly convex, and is functionally the outer hinge. The lower moiety is the middle piece. On its outer face there are several large sessile glands which may be considered a releasing mechanism, as it has been found by experiment that activation is procured readily by touching the door at this point rather than elsewhere. It is peculiar that the middle piece is strongly bent transversely, so that, when relaxed, and viewed sagittally, the door is flexed into an S-form. This flexure contributes to the firmness with which the middle piece engages the middle zone of the threshold.

The threshold is very deep and presents two very marked regions, the outer, clothed with the velum, and in the sagittal section nearly straight, and an inner, bearing the middle and inner zones of the pavement epithelium (the latter quite narrow), facing the interior of the trap, and bent at an angle of some 40–50° with the outer zone surface. Since this inwardly facing surface bears the middle zone of the pavement epithelium furnishing the surface of contact for the middle piece of the door, we are faced with conditions apparently contradictory to the mechanical necessities, more or less completely satisfied by previously considered types. It may be said at once, however, that those mechanical conditions exemplified by *U. cornuta* and *U. caerulea* are fulfilled also in *U. monanthos*. This is brought out by the study of door postures when the trap is set and after activation[1].
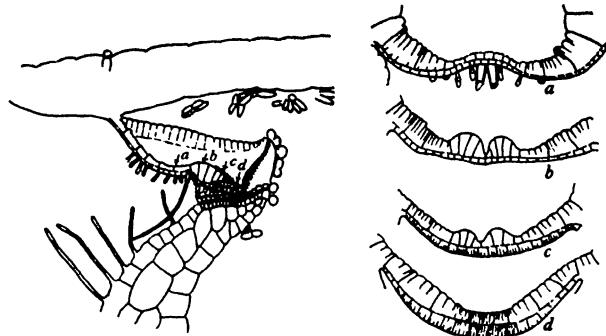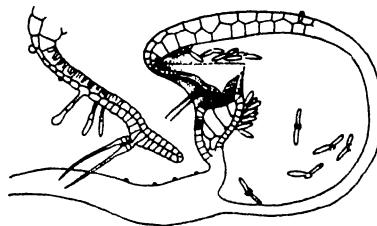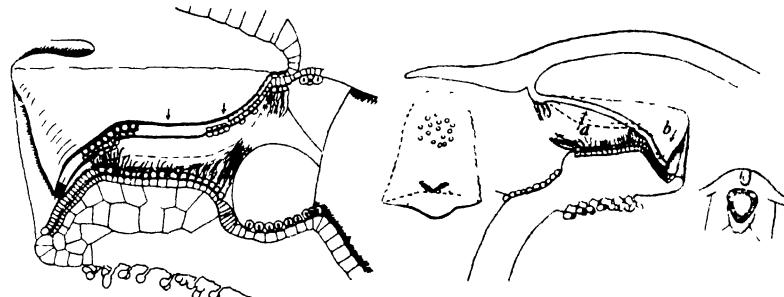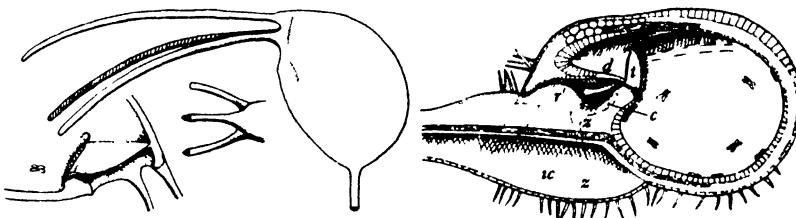
Fig. 32

Fig. 33

Fig. 34

Fig. 35

Fig. 32. *U. Kirkii.*
Fig. 33. *U. nana.* ·
Fig. 34. *U. Menziesii* (left) and *U. monanthos*, the latter showing the posture of the door before and after actuation. Door, *en face*, left and entrance, with circular velum, on right. *a*, direction of water pressure; *b*, direction of thrust of outer hinge.
Fig. 35. *U. tubulata* (left) and *Polypompholyx.* *t*, threshold; *d*, door; *r'*, ridge along upper side of the stalk; *z, z*, patch of bristles (see Fig. 3); *ic*, intercellular space; *c*, floor of antechamber.

This species, because of the form of the trap, betrays its door postures very clearly, and these are shown in Fig. 34, made from photographs. In the set condition, the profile of the door is quite convex, sweeping backward and downwards to meet the threshold at the transverse bend, beyond which the middle piece is applied to the inwardly facing middle zone of the pavement epithelium. The curvature of the door is now continuous, the bend of the middle piece being obliterated, thus contributing its resulting rigidity to the necessary, if unstable, equilibrium, to which the lateral thrusting outer hinge also contributes, as in *U. cornuta*. This factor is the the more efficient because of the narrowness of the door. The watertightness of the whole is assured by the velum clothing the bend of the threshold. Guarding the outer entrance the velum takes a circular form, and this appears to contribute to the stability of the set condition by plugging the angles of the door and walls (Fig. 34). When one looks straight into the entrance, this part of the velum shows as a nearly circular veil with a rounded aperture, through which the prey has to enter to reach the door surface.

Activation of the trap is procured experimentally by applying a very gentle touch of a glass probe to the door surface at or near the point at which the lower group of glands is situated. Even a strong impact of the probe on the upper part of the surface has no effect. This is as in *U. cornuta* and *U. caerulea* whose action is quite as in this species. On the return of the door it assumes the relaxed posture, in which the upper moiety is strongly convex, as indicated by the broken lines in Fig. 34. The resetting has been observed to require a half-hour or more, but is as prompt approximately as in *U. vulgaris*. It is evident that in the alteration of curvatures during this process, the lower part of the door is cramped into position so that the contact of the door edge with the threshold is a very firm one.

The above, based on living material, must take precedence over my previous accounts (1932 *a*, 1933 *c*) which are faulty in not affording a correct description of the set and relaxed door postures.

The floating form *U. tubulata* evidently conforms to the type which has just been described (Lloyd, 1934), judging from the only material available, namely, rather badly preserved specimens in the British Museum, the type specimen used by v. Mueller. A single good trap available offered sufficient evidence to show the appendages (above described) and the sweeping curves of the door usual in the relaxed posture, together with the ridged threshold somewhat as in *Polypompholyx* (Fig. 35).

This genus, finally, has a trap which though differing so much, as we have already seen, from that of the genus *Utricularia*, has an entrance mechanism (Fig. 35) which agrees with the Australasian anchored species above mentioned, the free edge of the door overlying a transverse ridge formed by the threshold, but differs somewhat in the general shape of the door which is relatively broader and therefore would be more unstable under the conditions imposed by water pressure. As compensation, resulting in a greater firmness in posture, there is a much greater thickness of the upper moiety of the door. This is in striking contrast to the thin, parallel contours of the door of *U. monanthos*, etc. There is still some uncertainty about the size and character of the velum, though there is evidence of its presence; and that the trap works

in the same manner as all the others has been evidenced by observations made for me by Mrs Johnson, née Reed, of the University of Perth, who saw clearly the concave posture of the three sides of the living trap which changes to convex on the activation of the door.

If the plants which we have been considering are very uniform in general habit (to which of course there are a few notable exceptions, especially in the large and impressive South American species, *U. reniformis*, *U. Humboldtii*, etc.), and even if this uniformity approaches monotony, they have succeeded in attaining a diversity of structure of their traps which presents intricacies parallel only to those seen in the flowers of the Orchidaceae, in which regard, however, the genus *Utricularia* finds its greater degree of uniformity. The late Prof. Goebel, shortly before his death, acknowledged this in personal intercourse. Through his kind generosity I was able to make free use of the rich treasures of his collections which included many taken by his own hand. Had it not been for this liberality on Prof. Goebel's part, this work would hardly have progressed at more than half the rate.

VIII. SUMMARY.

The present status of our knowledge of the form and structure of the plant body in the genus *Utricularia* (incl. *Biovularia* and *Polypompholyx*), apart from the formal morphological point of view, is briefly presented.

The account embraces (*a*) the period of embryological development, during which anatomical-nutritional relations are prominent; a very peculiar feature is the abstriction of the root pole of the embryo by the endosperm; (*b*) the form of the rootless definitive embryo; and (*c*) its behaviour during germination, of which there are several types.

Then follow descriptions of the various biological forms of the so-called leaf, stolon, tubers and resting buds.

The various forms of the trap (bladder) are described. While all have the same fundamental structure, the general form may be extremely simple in bearing no appendages, or may be equally complex in having numerous appendages of various kinds. The biological meaning of these is problematical.

The entrance mechanism of the trap is analysed and its mode of operation is shown. It is composed of two valves, the larger being the door, and the smaller being the velum, which overlies the edge of the larger. When the trap is set the door edge rests against an opposing surface of a ridge, the threshold, which impedes its swinging inwards, and in this position the door and the velum are mutually so adjusted as to be watertight. The setting of the trap is achieved by diffusion of water from its interior, a condition of unstable equilibrium being set up. The adjustment of the trap to this condition consists in the partial collapse of its side walls. Springing of the trap consists in disturbance of some sort of release mechanism consisting of bristles, larger or shorter trichomes, which thus distort the door, allowing the higher outer water pressure to swing it in. The entering water current carries in the prey if suitably placed.

Although all the traps known are alike in principle of action, there is a considerable diversity of form and structure. The differences lie in the relative positions of the threshold and valves, and in the relative quantitative importance of their mutual thrusts. Such differences are expressed in an extended variety of form of every part.

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PROTOPLASMIC REORGANISATION AND ANIMAL LIFE CYCLES

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CONTENTS.

I. INTRODUCTION.

THE present decade of 1930–40 has especial historic significance for biologists, since it completes a century of biological advancement that began with such notable events as Robert Brown's discovery of the cell nucleus in 1831 and Schleiden and Schwann's enunciation of the cell theory in 1838-9. These familiar events are of first importance here because of their direct bearing on our present-day knowledge and problems of life cycles.

Other events of that memorable decade of a century ago, which are quite as important in this connection, include the discovery of protoplasm by Dujardin (1835) and the publication of a pioneer monograph on the Infusoria by Ehrenberg in 1838. The latter work incorporated previously published claims of Ehrenberg that were more or less directly the occasion of Dujardin's significant discovery. The primary aim, however, in the writing of his paper "Sur les prétendus estomacs des animalcules infusoires et sur une substance appelée sarcode," was not to announce his discovery of sarcode but rather to refute the polygastric theory of Ehrenberg.

According to Ehrenberg, not only did all animals, however large or small, carry on fundamentally the same functions but also for each function there must be somewhere, and very probably discoverable, an organ performing that function. Thus the protozoan nucleus was a testis, the contractile vacuole was a seminal vesicle, and most certainly these *Infusionsthierchen* had stomachs, even as many as 200, all united into a continuous alimentary tract. Hence, these minute creatures were to be regarded as complete organisms.

Against these claims, this polygastric theory, Dujardin was diametrically opposed. He denied the existence of a continuous digestive canal and other comparably definite organs, and he insisted that the comparison between these microscopic animals and the larger forms was one of striking differences rather than similarities, the contrast of simplicity and complexity. It was in evidence of this simplicity that

he studied and described with remarkable accuracy the homogeneous, hyaline, glutinous, and viscid substance to which he gave the name sarcode.

This comparison between microscopic and macroscopic forms of life, or, since von Siebold's time (1845), between unicellular and multicellular organisms has persisted as one of our major biological problems, and controversies on this comparison did not end a century ago with Dujardin and Ehrenberg. The published claims in our biological literature have varied all the way from regarding the protozoan cell as the equivalent of a metazoan cell to the declaration some years ago by Dobell (1911) that the protozoan cell is not a cell at all, so that we should regard the Protozoa as non-cellular organisms.

Both of these extreme views, as well as the Dujardin-Ehrenberg controversy, appear to illustrate an inadequate perspective which is traceable, as I believe, to an unwholesome emphasis on the structure-function aspect of living things without due regard to their historical relationships. In common parlance, a cell is a cell to be sure because structurally it has nucleus and cytoplasm and performs the elementary functions. But to identify Protozoa as cells for these reasons and for these reasons only, or to deny their cellularity even though they possess nucleus and cytoplasm, and then omit from these considerations the individual and racial history of the Protozoa denotes a need of larger perspective for want of which, particularly in this day and age, no investigator of these miniature forms of life can adequately interpret the results of his labours.

II. PROTOPLASMIC REORGANISATION.

About 25 years after the Dujardin-Ehrenberg controversy, Stein (1859) discovered a phenomenon in dividing hypotrichous ciliates which should help us not only to understand the Protozoa better but also to relate more completely their life cycle with that of the Metazoa. Stein first observed in *Stylonychia* that during binary fission all the motor organelles, such as cirri and membranelles, were gradually resorbed in the mother organism and simultaneously a new set emerged and replaced the old in each of the resulting daughters. This author had previously (1854) described the complete disappearance of the ciliary apparatus of *Epiclintes* during its encystment, which agreed with similar observations of other workers. But no special significance appears to have been attached to these findings. Some years later Engelmann (1862) recorded the outgrowth of new cirri and disappearance of the old in conjugating *Euplotes charon* and in 1889 Maupas described the same procedure in conjugating pairs of *E. patells*.

No marked attention, however, was given to this phenomenon until the beginning of the present century when Wallengren published in 1900 and 1901 the results of his classical studies which confirmed and considerably extended the observations of the earlier workers. This author described in careful detail the extra-nuclear changes that occur during fission and conjugation of several hypotrichous ciliates, including *Euplotes harpa* and *Uronychia transfuga*. In each instance he observed that all locomotor organelles of the dividing mother organism were gradually resorbed and simultaneously there appeared ventrally in the plane of

fission hyaline areas from which grew pairs of minute buds, the *Anlagen* of the new cirri (Fig. 1 *A*, *B*, *C*). Each bud of a given pair, as it elongated, proceeded to migrate, one anteriorly and the other posteriorly where it assumed its normal position in the anterior and the posterior daughters respectively. Similarly the membranelles and undulating membranes were replaced by a new set whose origin was likewise *de novo* from the cortical protoplasm. In like manner, each member of a conjugating pair of these several hypotrichs resorbed all of its locomotor organelles and reformed *de novo* a new set.

Following Wallengren's careful studies of this profound reorganisation process, various workers have since described a similar procedure for *dividing, conjugating, encysting* and *regenerating* ciliates, including other hypotrichs, heterotrichs and a
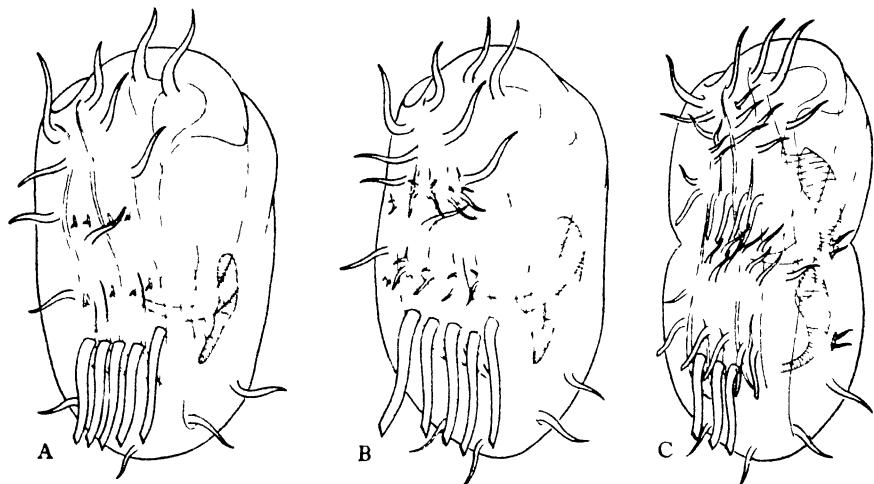
Fig. 1. Three stages of cytoplasmic reorganisation of *Euplotes harpa* during binary fission. Note the outgrowth and migration of new cirri and membranelles which will replace the resorbing old cirri and membranelles in the resulting daughter organisms (after Wallengren).

few holotrichs. As illustrative, in 1910 Griffin observed this phenomenon in dividing *Euplotes worcesteri*, and in 1918 Yocom found it to occur in *E. patella*. In 1925, MacDougall described comparable reorganisation changes in the oral basket, oral cilia and probably the body cilia of the holotrich, *Chilodon uncinatus* (Fig. 2 *A*, *B*, *C*, *D*), and more recently Lucas (1932) observed a similar, although rather unique type of ciliary reorganisation in another holotrich, *Cyathodinium piriforme* (Fig. 3 *A*, *B*, *C*, *D*, *E*, *F*). In a recent issue of the *Archiv für Protistenkunde*, Poljansky (1934) gives a detailed description of conjugating and encysted *Bursaria truncatella* which clearly demonstrates for this heterotrich that all extra-nuclear organelles, including the body cilia, undergo resorption and a redifferentiation. This last work represents probably the most complete account of the process since Wallengren's publications.

For this same ciliate (*B. truncatella*), Lund in 1917 observed similar changes during regeneration. Other studies on these changes during regeneration include

those of Dembowska in 1925 and 1927 on *Stylonchia*, *Euplotes* and *Uronychia* and of Taylor in 1923 and 1928 on *E. patella* and *U. uncinata* (Fig. 4 *A*, *B*, *C*, *D*).

It thus appears that for the ciliates the process of protoplasmic reorganisation is widespread and may very likely be found to be universal.

For several flagellates, a comparable phenomenon has been described. Citations
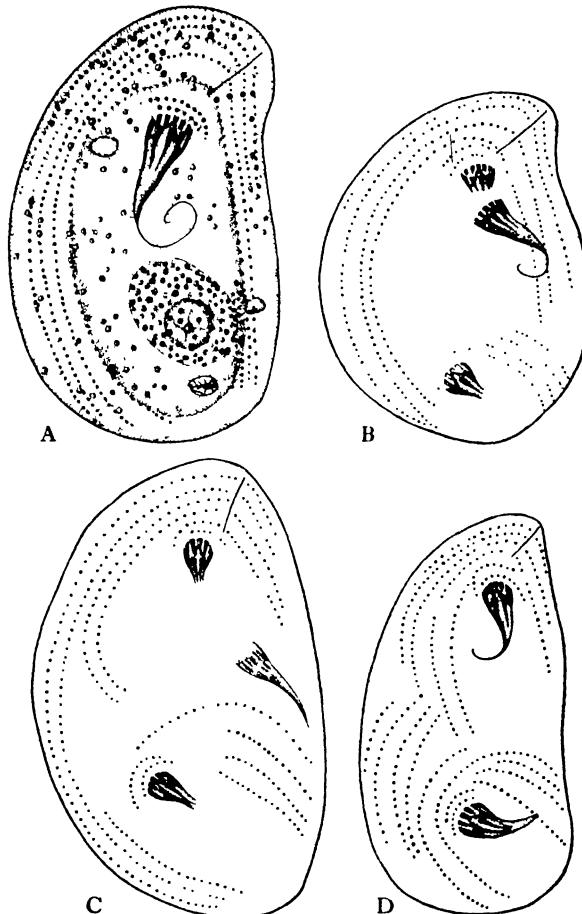
Fig. 2. Stages of reorganisation during the binary fission of the holotrich, *Chilodon uncinatus*, showing the resorption of the old oral basket and the origin *de novo* of the two new oral baskets, one for each daughter (after MacDougall).

to the more recent of these descriptions may be found in Kirby's publication of 1931 on, "Trichomonad Flagellates from Termites." While it is yet uncertain as to how complete and general this phenomenon may be for the extra-nuclear organelles of the Flagellata as a group, it is of interest in this connection to note, quoting Kirby (p. 237), that "The staborgan of *Peranema* (Brown, 1930) and of *Heteronema* (Rhodes, 1926); the entire flagellar, axostylar, and parabasal apparatus
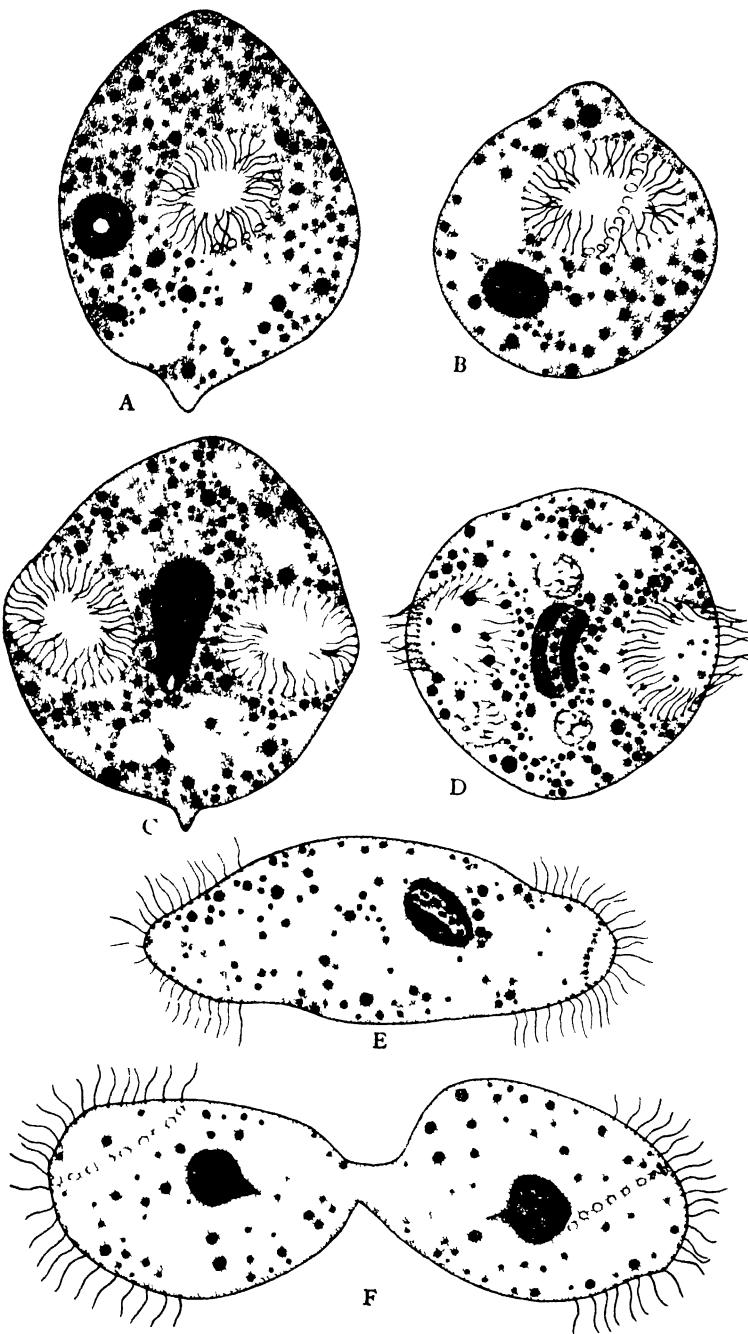
Fig. 3. An unusual origin of body cilia found in a holotrich, *Cyathodinium piriforme*, during binary fission. The new cilia are derived within a vacuolar cavity which divides, and later the cilia become everted to replace the resorbed cilia of the mother organism (after Lucas).
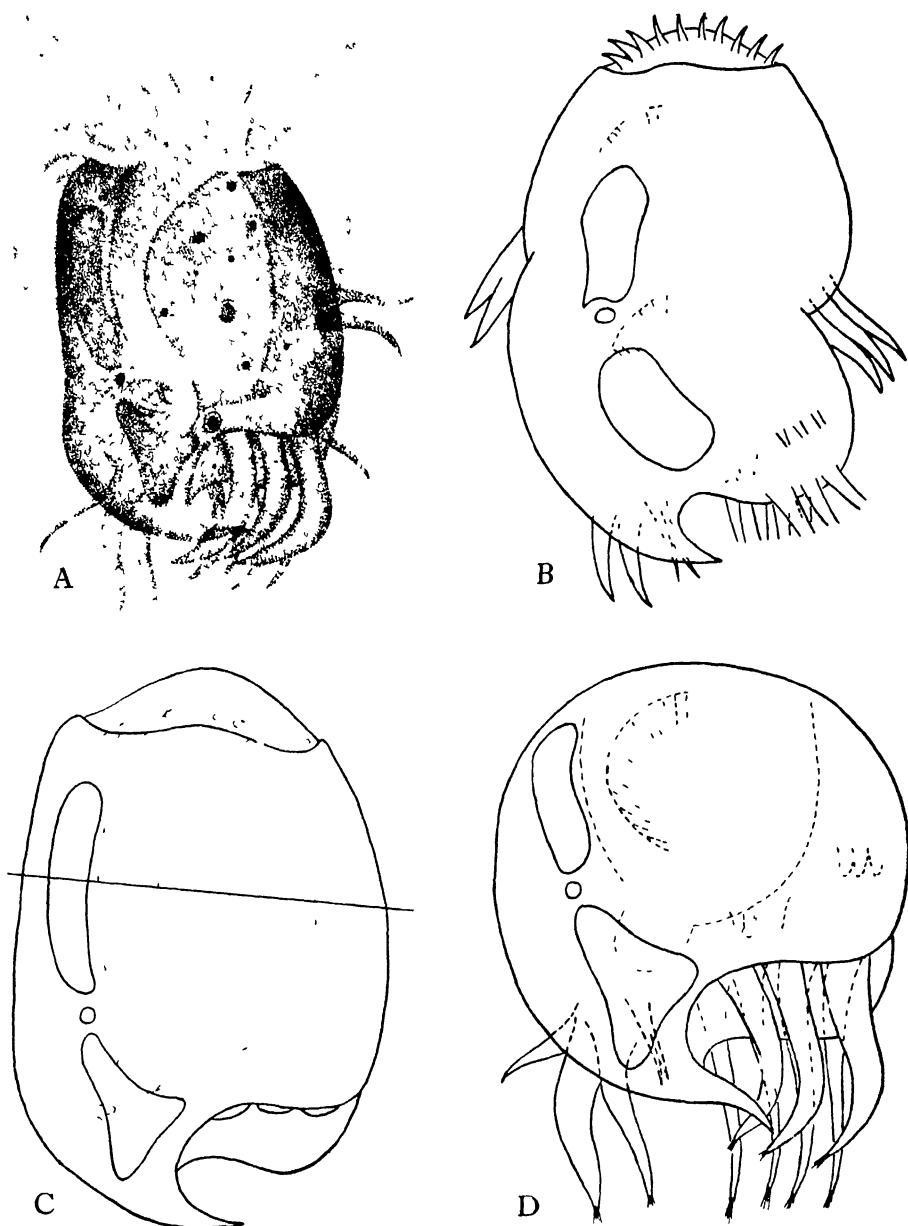
Fig 4. Resorption of the locomotor organelles of *Uronychia uncinata* during binary fission and regeneration.
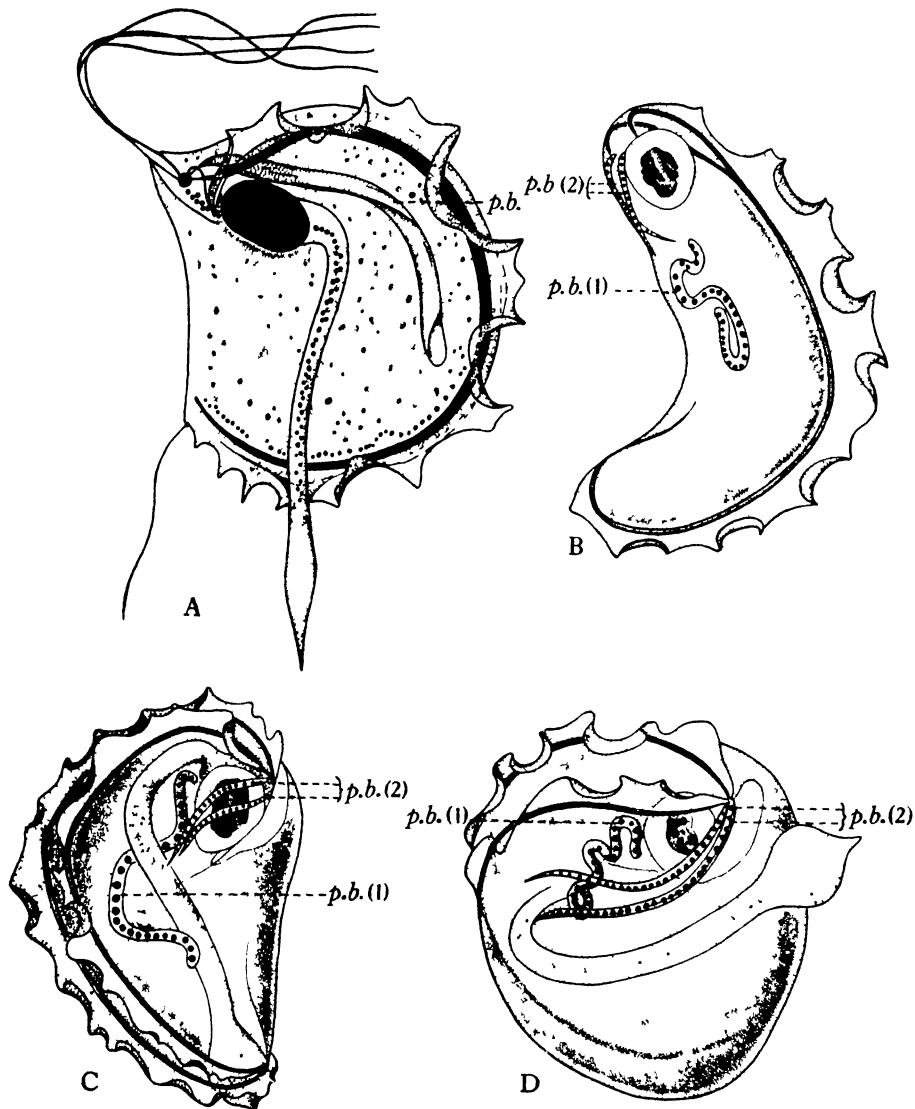
Fig. 5. Four views of a trichomonad flagellate, *Trichomonas termopsidis* B, C and D are stages of binary fission during which the parabasal body (p b 1) undergoes resorption and the new parabasals (p.b. 2) are derived *de novo* (after Kirby).

of *Lophomonas*; the flagellar apparatus and cyto-endoskeleton of *Kofoidia* (Light, 1927); and the flagella, blepharoplasts, axostyle, and fibrils of *Proboscidiella* (Kirby, 1928) and of *Oxymonas* (Connell, 1930)" undergo resorption to be replaced in the daughter organisms by newly differentiated structures (Fig. 5, *A, B, C D*).

Quite recently Reynolds (1934) described in a monad, *Monas vestita*, the re-

sorption of the two unequal flagella during binary fission, the shorter flagellum disappearing first (which made observation the more clear and certain) followed by an outgrowth of two new flagella for each resulting daughter.

How widespread this phenomenon may be, as described for various Protozoa, is yet to be determined; especially so with regard to the manner in which it occurs. It is possible that further researches will disclose devious ways in which it is carried out, or that in some Protozoa it is carried out only in part as compared with hypotrichous ciliates and some holotrichs in which it can be so clearly observed. But this variability will most likely be found, if found at all, to hold only for the cytoplasmic differentiated structures. For nuclear behaviour, we already know that reorganisation is fundamentally similar in all the ciliates and that in the rhizopods and flagellates its various manifestations during fission are interpretable in a fairly comprehensive series which show much in common and which appear to exhibit vestiges that may represent the course of evolution of the nucleus in its present, most primitive form.

III. LIFE CYCLES.

We may now turn to certain considerations of the significance of these protoplasmic changes during the life cycle of the Protozoa which bear especially upon apparent discrepancies between their life cycle and that of the Metazoa. Obviously, we are faced here with the general problem of the essential relationship between the Protozoa as a group and the Metazoa as a group. These two groups structurally seem to share in common the feature of cellularity which has so frequently served as the basis of their comparison. As Dobell (1911) has pointed out, however, this comparison is not justifiable if we recognise the protozoön as an organism functionally quite comparable with the metazoön as an organism, for if a comparison holds fully for two things, certainly it cannot hold for one of those things and only part of the other. Hence the concept of cellularity is, according to Dobell, applicable only to the Metazoa. The Protozoa (or preferably Protista), accordingly, are to be regarded as non-cellular with an altogether different kind of organisation which segregates them exclusively from multicellular plants and animals.

What appears to be the major difficulty in comparing the Protozoa with the Metazoa arises, it seems to me, out of limiting our considerations chiefly to their structure-function relationships. Thus, in terms of the cell as the unit of structure and function, we are wont to compare *fully* developed one-celled organisms with *fully* developed many-celled organisms. And this comes to be pretty much a fixed picture which, as a result, tends to subordinate or leave out of account the more fundamental fact that antecedent to the fully developed protozoön and metazoön, there is a developmental history, an evolutionary history, the product of which is their differentiated structure and function.

Platitudinous as this may at first appear, it is none the less, I believe, a crucial, indispensable point of view because it seeks to emphasise the time factor, the sequence of events, in life phenomena, and this is of paramount importance.

From this historical viewpoint, our basis of comparison of Protozoa with Metazoa is primarily not cellular structure, as between the unicellular and the multicellular, nor is it primarily cellular function, attributing to one-celled animals all the functions characteristic of many-celled animals. Instead, our fundamental comparison of these two great groups has to do first of all with their genetic history, which includes both their ontogeny and their phylogeny. The essential features of metazoan ontogeny are well known. It is only convenient here to recall that each such organism conspicuously begins its individual history as a single cell—the ovum. And we may for the present omit the phenomenon of amphimixis (the union of two gametes at fertilisation) especially since we know that this union is not indispensable even for the Metazoa.

Furthermore, we are more or less familiar with the previous history of this ovum. We know that it is the resultant of a sequence of events which extend back throughout the generations of time; and it is through this sequence, this cellular line of descent that, counting time backwards, ontogeny meets, unites with, and is lost in phylogeny—much as the limb merges and disappears into the trunk of the tree.

Or, reversing this moving picture, that is, counting time forward—as Nature chooses to count it—we observe ontogeny emerging from the main phylogenetic trunk, and through that remarkable visible sequence of events (the phenomenon of developmental differentiation), our primordial cell becomes an oöcyte, an ovum, a blastula, a gastrula, and on finally to the adult stage of a fully developed metazoön.

Metazoan ontogeny, or life history, has to do, then, primarily with a dynamic process, viz., differentiation which begins with a preformed cell, the primordial cell, whose protoplasm came to be differentiated during phylogenetic history into cytoplasm and nucleus. We may, I think, rightly suppose that this process of protoplasmic differentiation *was* during phylogeny, as it *is* during ontogeny, fundamentally epigenetic in character, unless we conceive the cell to have sprung full-fledged like an Athena from the head of Zeus.

At any rate, it does appear that the *process*, differentiation, rather than the product, cellularity, ought to be of first importance in all our fundamental considerations of genetic history. If so, then it would seem that we have in that phenomenon of differentiation a proper basis of comparison between metazoan genetic history on the one hand, and protozoan genetic history on the other. For each member of these two great groups begins its life history typically as a *preformed cell, a primordial cell which we may characterise simply as the stage of minimal protoplasmic differentiation*. At that primordial cell-stage obviously both metazoön and protozoön are, in the strictest sense of the term, truly unicellular organisms, each of which in subsequent ontogeny *behaves as an organismal unit*.

Out of that primordial stage come to be differentiated, epigenetically, highly diverse structures with specific functions which for the Metazoa are derived during *numberless* mitotic divisions, while, on the contrary, for the Protozoa these organic structures and functions are derived primitively during *one* mitotic division, and are re-derived at each succeeding mitotic division.

Thus on the basis of this phenomenon of organic differentiation we come to

observe the essential and striking difference between protozoan and metazoan ontogenies, and, correspondingly, we may regard this as the fundamental distinction between the Protozoa as a group on the one hand and the Metazoa as a group on the other, in that the former, unlike the latter, retains the capacity to dedifferentiate, that is, to revert back to its primordial stage. This it *tends* to do every time it undergoes mitotic division (or fission), and similarly at each encystment and during each conjugation and during regeneration. Metazoan ontogeny, on the contrary, proceeds toward a fixed and irreversible state of differentiation. In the lower phyla and during the early stages of ontogeny in the higher phyla this capacity to dedifferentiate is manifest in the results of egg-fragment studies, of blastomere isolations and of early and later regenerative phenomena varying, we know, according to the species and depending upon the tissues or parts concerned in individual organisms. But the *tendency* in metazoan ontogeny is clearly toward irreversibility which eventuates in disintegration and death. Thus the Protozoa, retaining that capacity of dedifferentiation and redifferentiation, or in a word the capacity of reorganisation, may be regarded as potentially immortal beings.

IV. CYTOPLASMIC *VERSUS* NUCLEAR REORGANISATION.

There remains one other consideration regarding this phenomenon of protoplasmic reorganisation which, as a corollary, might properly constitute the concluding paragraphs of this brief survey. It may be noted that the thesis here proposed places special emphasis not only upon the genetic history of both Protozoa and Metazoa but also upon their organismal unity. This unity is clearly manifest in metazoan ontogeny as demonstrated especially in the results of modern embryological experimentation.

But it is in the ontogeny of the protozoan organism that this unity is, it would seem, particularly evident and significant. For there we have to do more directly with the products of phylogenetic differentiation, viz., the cytoplasm and nucleus. In so far as ontogenetic differentiation concerns the cytoplasm, the observations and recent experiments cited above point toward a unified behaviour throughout the cytoplasmic mass. Thus, the removal of a portion of that differentiated mass, or even the basal injury of a cirrus, induces a general and profound change that involves the resorption of all extra-nuclear organelles and the outgrowth of a new set.

But the question now arises: On the basis of this organismal unity are we to suppose that the fundamental nature of this profound reorganisation is peculiar to the cytoplasm? It appears that such an assumption for the more primitive Protozoa, at least, may prove untenable, and while much further investigation is here required, I should like to venture the suggestion that their nuclear reorganisation may be found to be altogether comparable with cytoplasmic reorganisation in that it may involve the resorption of the differentiated structures such as the nuclear fibrils, the nucleoli and the chromosomes, and the redifferentiation of any and all of these protoplasmic structures.

The essential differences between nuclear and cytoplasmic reorganisation may

have to do with the discrepancy in their periods of phase when dedifferentiation ensues. Thus, while some nuclear components may dedifferentiate at the beginning of the interphase, cytoplasmic components on the other hand may not start to resorb until near the close of the interphase. Accordingly, the partial synchrony of cytoplasmic dedifferentiation and redifferentiation does not appear in nuclear reorganisation where a "resting stage" ordinarily intervenes. Moreover, there may also occur discrepancies in the completeness of both cytoplasmic and nuclear reorganisation, particularly in so far as it concerns the continuity of differentiated parts. For example, macronuclear resorption may not ensue until conjugation or endomixis, although visibly profound changes do occur in the macronucleus of many ciliates during fission, cystment, and regeneration. Thus the stage of minimal differentiation or maximal dedifferentiation, that is, the primordial cell stage, may not be attained for the Ciliophora and similarly for other conjugating Protozoa until that gametic period in their life cycle is realised. It is of interest in this connection to note, however, that Woodruff (1925), for laboratory cultures, questions the indispensability of either conjugation or endomixis, "...to carry on the stream of life uninterrupted".

V. SUMMARY.

Comparisons of the Protozoa and Metazoa have emphasised their structure-function relationships without due regard to their historical relationships. Thus cellularity has been a common basis for this comparison as applied to unicellular organisms on the one hand and multicellular organisms on the other, equating in each group the cell as the unit of structure and function; or, on the contrary, denying that this comparison holds since Protozoa are to be regarded as non-cellular organisms.

It is proposed that genetic history, including both ontogeny and phylogeny, is the essential basis for this comparison. Since organic differentiation is the process by which the cell originated in phylogeny and each organism develops in ontogeny, maintaining its organismal unity throughout, it is suggested that primarily this process, protoplasmic differentiation, and secondarily its product, the primordial cell, should be given first importance in fundamental considerations of both protozoan and metazoan genetic history.

Protozoa as well as Metazoa begin their life cycle as a primordial cell which is the stage of minimal protoplasmic differentiation. From this primordial stage comes to be differentiated epigenetically diverse organs with specific functions, derived for the Metazoa during numberless mitotic divisions but for the Protozoa during one mitotic division and are rederived during each succeeding division. Thus, Protozoa retain the capacity to reorganise, that is, to dedifferentiate and redifferentiate, which they tend to do during fission, conjugation, endomixis, cystment, and regeneration; and are accordingly potentially immortal. Metazoan ontogeny, however, proceeds toward a fixed and irreversible state of differentiation which eventuates in disintegration and death.

It is postulated further that protoplasmic reorganisation involves both cyto-plasmic and nuclear structures which alike undergo a redifferentiation following dedifferentiation toward a primordial stage which represents the initial stage of the life cycle. While these changes may differ, with respect to the cytoplasm and nucleus, in the time, manner, or completeness of their occurrence; it is suggested that their visible manifestations have to do with dynamic processes which are fundamentally the same.

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THE EFFECT OF GENES ON THE DEVELOPMENT OF SIZE AND FORM

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CONTENTS.

I. INTRODUCTION.

THE advancement in our understanding of heredity, which has come about so rapidly in the last thirty years, has been accompanied by a progressive narrowing in the field of interest of those who have studied its questions, and by increasing restrictions and refinements in the definition of its essential problems. The move toward definiteness and limitation was begun by Mendel, who focused his attention on *single*-character differences between individuals. When the laws governing these became apparent, the focus underwent a further narrowing to include only the particles in the reproductive cells which were responsible for the characters. The decisive results which we owe chiefly to the influence of Morgan and his school justify these methods, for now we can look with considerable confidence upon these particles, the genes, as the fundamental units of heredity.

This view rests upon evidence derived chiefly from experimental analysis of the heredity of morphological characters which differentiate members of the same species. Characters in which species themselves differ are also probably referable to genes in the same sense, but the analyses are as yet incomplete and inconclusive. There is no doubt, however, that the vast majority of hereditary characters which have been carefully studied are due to gene differences, for in only a very few cases, and these of a special nature, has inheritance through the cytoplasm been proved. It is therefore a fair inference that where differentiating characters are transmitted, genes are the units by which this is accomplished, whether individual Mendelian analyses have been made or not.

Genes appear to be distinct chemical entities transmitted as parts of chromosomes. Each retains a high degree of stability in its passage from generation to

generation and reproduces its specific structure faithfully at each mitotic division. Rarely the structure undergoes a slight change (mutation), as inferred from the fact that the gene now has a different effect and thereafter retains its new characteristics in reproduction and development. The study of such changes in the genes has occupied a prominent place in genetics during the last few years, chiefly because it has been found possible to induce mutations and to study the process by quantitative experimental methods. These advances have served to narrow the focus still further, until it now rests upon the structure of the gene and upon the changes which may take place within it.

In this process of concentrating attention upon the gene itself, the character, by which alone the gene may be identified, has frequently been relegated to the position of a mere tag or marker for the gene, and problems of wide biological importance, having to do with the mechanisms through which the gene affects development, have been relatively neglected. Thus it has come about that the theory of the gene has been stated without reference to development (Morgan, 1929). But the problem of hereditary differences among organisms does not cease when the mechanism of transmission has been solved. It may be that its ultimate solution lies chiefly within other fields, notably those of the embryologist and experimental morphologist, but the very unification of our views of the organism which the concept of the gene has brought indicates how difficult it has become to maintain the traditional boundaries between fields of work. A utilisation of the methods and ideas of both genetics and development in a concerted attack is now necessary for the solution of the general problems of heredity.

Although attempts to construct a unified theory of heredity have often been regarded as premature, because direct evidence of the effects of genes on development is fragmentary as compared with that relating to their transmission, they have not been without profit, as shown by the pioneer work of Goldschmidt (1927, 1932) in outlining a general physiological theory, from which many concepts useful for future work may be derived.

The fact that genes affect development is obvious and firmly established; how they do it admits of varying interpretations, none of them conclusively proved. It is clear that they act in general through causing changes in the cytoplasm, for differences among cells and parts arise in normal development only in the cytoplasm, the genes and chromosomes of all cells being similar by reason of the exact equational nature of the nuclear divisions. How the genes affect the cytoplasm is thus the ultimate problem. We shall not attempt to formulate any general answer to this here, but instead we shall review some of the recent work which has attempted to describe in morphological terms the developmental history of certain characters of size and form which have been shown to depend upon specific genes. Such direct examinations of development in genetically analysed material have not been made in many cases, and we have chosen, for the most part, those which are relatively recent and with which we have had some personal experience. In thus confining our discussion chiefly to genic [effects on total body size, sizes and proportions of parts, shape, pattern, and in general those aspects of the organism which can

be observed by the methods of gross morphology and histology, we do not propose to substitute morphological for physiological explanations of what is essentially a dynamic process. Before any fundamental study of gene action can be made, however, it is first necessary to obtain detailed descriptions of the visible changes which occur in development, and such should be the first goal of developmental genetics.

In attempting to distinguish between the effects of various genes it is important not to fall into the habit of speech and thought which dominated the early years of genetic science. That implied a simple cause-and-effect relationship between "unit factor" and "unit character." Such a conception would lead us to speak of "size genes" and "colour genes" as in separate categories each leading by itself to a different end result. It is quite evident now that most genes have many effects, and that any categories we may make at this time are probably artificial ones, defined by those effects which are most obvious. A gene may well have certain general effects on all cell processes and certain special ones, determined in part by the chemical nature of the specific gene and in part by its relationship in time and space to the reactions set in motion by the other genes and by the environment. A "size gene" may thus exert at one time and under one set of conditions a general effect upon size, at another a localised or special effect, and may also influence various other traits such as colour or texture. Our problem at the moment is to describe the character and order of occurrence of any effects, clearly attributable to genes, which can be studied quantitatively.

The various effects which genes may be expected to have upon developmental processes can perhaps be classified most readily into effects upon size—the total amount of growth attained by organ or body during its development—and effects upon form and structure resulting from the changes which accompany this growth process. The chief purpose of our inquiry is therefore to determine to what extent these two major categories of traits—those of size and of form—are under genic control and in what manner this control is exercised.

II. GENIC CONTROL OF SIZE.

Ever since Mendel's classic experiments it has been evident that certain quantitative differences are directly related to gene differences. The most conspicuous "unit character" which Mendel found was concerned with plant height, and the fact that in the F_2 from a cross between a tall and a dwarf race there was a sharp monohybrid segregation into tall and dwarf types clearly shows that this difference in amount of growth is determined by a pair of genes. Later investigations, notably by Keeble and Pellew (1910) and de Haan (1927), showed that inheritance of stature in peas is not quite so simple as Mendel thought it, and that plant height is also affected by other genes than the ones he observed. The distinctness of these other genes is shown by the differences in their effects. Thus the type of plant with the gene for "slender" vines found by de Haan is readily distinguishable both externally and histologically from the "tall" type described by Mendel. There is a clear distinction between a gene controlling internode length and one controlling number

of internodes, as described by Keeble and Pellew, though both markedly influence plant height.

In many other species of plants and animals differences in the size of the individual or of its parts may be related to a single gene difference. The indeterminate habit in beans, resulting in a tall, climbing plant, shows monohybrid segregation in crosses with the dwarf bush form of limited growth; and the tall type in tomatoes, portulacas, and many other plants may differ by only a single gene from the more compact dwarf form.

A similar situation has been found in animals where the difference between "giant" or "dwarf" races and the normal type seems to be due to single genes (*e.g.* *Drosophila*, mice). Analysis of the effect of the "dwarf" gene in mice by Smith and MacDowell (1930) has shown that its chief effect is to impair the production of a growth hormone in the anterior pituitary gland; and this leads to other abnormal changes such as sterility, showing that dwarfism may be the indirect result of other physiological effects of the genes, some of them of an abnormal character.

Although such simple instances of genic control over growth are not uncommon, it is nevertheless true that in the majority of cases, differences between two types in the amount of growth are difficult to analyse in Mendelian terms or to refer to the action of particular genes. In such cases there is no simple segregation after crossing. Evidence is steadily accumulating, however, that these more complex instances differ from the simpler ones only in the larger number of gene differences involved. This conclusion, first suggested by Nilsson-Ehle and East twenty-five years ago, is of considerable importance for our problem, since, if true, it makes it possible to consider growth in general as under genic control.

This multiple-factor theory of the inheritance of quantitative characters is now supported by at least five lines of evidence. (1) In the F_2 from a cross between two pure types differing in a quantitative trait, the variability is almost always greater than that of the F_1. (2) The F_3 progenies grown from various parts of the F_2 range are genetically distinct from each other, showing that the increased variability of the F_2 is due to segregation. (3) In a number of plants and animals quantitative traits show linkage with others, the genes for which have been traced to known loci in the chromosomes, thus indicating a similar genetic basis for both kinds of traits. (4) The evidence for the hypothesis of genic balance indicates that each chromosome has an influence upon many quantitative traits. In each of the twelve "primary" mutants of *Datura stramonium* (Blakeslee, 1921) one of the twelve chromosomes of the species is represented by three members instead of two. In each mutant there are significant and typical differences in many quantitative characters, indicating that each chromosome contains specific genes affecting such characters. It is impossible to explain the character effects of ploidy, heteroploidy, and chromosome deficiency without the assumption of multiple genes affecting growth. (5) The phenomenon of heterosis or hybrid vigour in the heterozygous offspring from a cross between two races can best be explained on the assumption that growth vigour results from the cumulative expression of many different genes affecting size (Jones, 1917). The accepted explanation of the effects of inbreeding as conse-

quences of Mendelian mechanisms of segregation and assortment depends upon the same assumption.

Although the bulk of evidence thus seems to show that the size of an organism and of its parts (under constant environmental conditions) are controlled by genes, this conclusion is not at present universally accepted. Castle (1929) failed to detect clear segregation following crosses of races of rabbits differing in size or to find any evidence of linkage between genes influencing size and certain "colour" genes located in four different chromosomes. He concluded that although the growth rate is undoubtedly subject to inheritance there is no evidence that it is influenced by any gene in four linkage systems. He suggests that in mammals, at least, it may have its physical basis outside the chromosomes. Such an extreme position is difficult to maintain in view of the general evidence summarised above and particularly in view of Green's (1930, 1934) demonstration that, in the mouse, genes affecting size of the body and of specific parts show linkage with colour genes of known chromosome location. Castle's criticism, while it fails to upset the general thesis, does nevertheless emphasise the unconvincing nature of the evidence for multiple-factor control of size in higher animals and the need for caution in ascribing all such differences to genes without further proof. Such material offers special difficulties (obtaining pure strains, standardising conditions, and getting large numbers) in testing the general assumption. Since *hereditary* control of size characters is universally admitted, however, we may safely examine the steps through which such characters pass in development on the assumption that they, like most other hereditary traits, are controlled by genes.

Since the course of development of quantitative characters can be observed and measured by simple methods, it should be possible to determine, at least superficially, the means by which genetic differences in size are brought about. Three such possible methods of origin suggest themselves: (1) there may be differences in the *number* of cells; (2) there may be differences in the *size* of the cells; (3) there may be differences in size of body or of organ not dependent on either of these alone.

Differences in cell number. It is well recognised by both botanists and zoologists that most of the size differences among closely related organisms are due to differences in the number of cells of which they are composed. Cell sizes vary within much narrower ranges than body sizes. Whatever affects the rate or duration of cell division will thus influence the size of the whole, and one is tempted to attribute the role of genes in size determination to their control of the production of such substances as glutathione which may specifically stimulate mitosis. Indeed Gregory and Goss (1933) have found some evidence that the concentration of sulfhydryl is higher in large than in small races of rabbits.

Differences in cell number often make their appearance very early in development. Large-fruited races of tomato have many more cells in the earliest fruit primordium than small-fruited races (Houghtaling, 1935). Castle and Gregory (1929, 1931) have found that the developing eggs of rabbits of a genetically large race have more cells (blastomeres) than the eggs of similar age from rabbits of a

small race. This difference is present at 40 hours after fertilisation but not at 31 hours. It is therefore brought about by differences in the rate of cell division which express themselves very early in development, large-race eggs having the higher rate. The differences are inherited and hybrids from reciprocal crosses show intermediate rates. Painter (1928) had previously shown that later embryos (12 days) from large and small races have cells of the same size, showing that the differences in embryo size were due to cell number.

Another proof that differences in size due to cell number are under genic control is afforded by recent quantitative studies on the anatomy of the floral pedicel of *Datura stramonium* (Sinnott, Houghtaling and Blakeslee, 1934). Certain chromosomes (and thus their constituent genes) tend to increase cell number in the fundamental tissues, while other chromosomes tend to decrease cell number in these tissues. These differences largely determine the size of the pedicel.

Differences in cell size. Not all size differences, however, are due to differences in cell number, for there are clear cases of genetic differences in cell size. The best known of these are those associated with variation in the number of chromosomes. Boveri (1904), Gates (1909), the Marchals (1909), von Wettstein (1924) and others have reported marked differences in cell size among the various numbers of a polyploid series, cell volume increasing as the chromosome number rises. Body size is here roughly proportional to cell size, haploids tending to be dwarf and tetraploids developing the familiar giant or *gigas* form.

The relation of chromosome number to cell size may be a direct arithmetic one, as seems to be the case in spores, pollen grains, and certain other cells. The pollen grains of tetraploid plants of *Datura stramonium*, for example, are almost exactly twice the volume of those from diploid plants. In other cases, especially in highly vacuolate plant cells, the relation is a geometric one, the successively higher members of the polyploid series being constant multiples of the ones immediately below them. Thus in the cortex of the petiole of *D. stramonium* (Sinnott, Houghtaling and Blakeslee, 1934) each member of the series has a cell size for this tissue approximately three times that of the next lower member. The precise value of the exponent varies in different tissues and under different conditions.

That cell size is related to volume of chromatin rather than merely to number of chromosomes has been shown in a number of cases. Thus Lorbeer (1930), who measured chromosome volume in male and female plants of the liverwort *Sphaerocarpus*, found it to be significantly greater in the latter, and observed that the cells in the female gametophyte were larger than those in the male by about the same degree. Nawashin (1931), working with a series of species in *Crepis* differing in total chromosome length, which ranged from 42 to 112 relative units, found that the total length (and thus presumably the volume) of the chromosomes was directly proportional to the volume of the cells of the root tip.

In such cases as these, to be sure, it is amounts of chromatic material rather than genic differences as such which are related to cell size. In general, however, changes in the basic number of chromosomes may be assumed to be accompanied by proportional changes in the number of genes. Whether this is the cause of the changes

in cell size or whether the change is due in part to an altered nucleo-plasmic ratio is not yet certain.

That differences in cell size are not determined entirely by chromosome number or volume, but by genes influencing cell size, is shown by a considerable body of evidence. The cell size of tetraploids is often no different from that of diploids, showing that some factor other than chromosome number is concerned. More direct evidence is afforded by cases where two varieties or races differ significantly in cell size although their chromosome complements are apparently identical. Thus Levitskii and Kuzmina (1923) found that cell size was much larger in the leaves of fodder beets than in sugar beets. Miss Passmore (unpublished data) has isolated a pure race of *Cucurbita pepo* in which cells of the vegetative tissues (though not the pollen grains) are markedly larger than in most races of this species, although the chromosomes show no difference in size or number. When this race is crossed with smaller celled lines, the F_1 is approximately intermediate in cell size, and there is a pronounced increase in variability of this trait in the F_2, facts which indicate that cell size has here a genic basis. Similar evidence is also provided by the anatomy of the primary trisomic mutants of *Datura* previously mentioned. When certain chromosomes (such as $21 \cdot 22$) are present in trisomic condition, there is a general and significant increase in cell volume as compared with the diploid, and the effect of other chromosomes (such as $3 \cdot 3$) is to produce a general decrease of cell volume. These effects have no relation to the size of the particular chromosome concerned, and are presumably due to specific genes carried by these chromosomes.

An excellent example of the relation between cell size and both the quantity and quality of chromosomal material is provided by Dobzhansky's (1929) study of the size of wing cells in *Drosophila melanogaster*. From the offspring of a triploid female he secured individuals with varying numbers of X-chromosomes (1, 2, or 3) in combination with 2 and with 3 sets of autosomes. A close relationship was found between the total volume of chromosomes and the size of the wing cells. All portions of the chromosome complement were not equally important in this regard, however, since the small fourth chromosome had a much greater effect on cell size than did the larger Y-chromosome. Cell size was also markedly affected by particular genes independently of chromosome volume. The gene for miniature wing, for example, reduced cell size when present, and by this means produced its characteristic effect of reduced wing size.

General developmental differences. Although genes affect growth through controlling cell number and cell size, these two effects are not brought about in entire independence of each other, but their relations are co-ordinated through a third system which we have called the developmental schedule or pattern. Examination of the parts of the organism during development reveals a definite time schedule of relationships appearing early in growth which, more clearly than any of the elements of cell size or cell number through which it is expressed, seems directly under genic control and results in absolute size differences which are not dependent upon either size component alone.

In general, cell division, resulting in increased cell number, predominates in the

early phases of growth, and differences in cell size and form arise only in the later ones. The relative duration of these two phases and the extent to which they transgress upon one another are elements in a specific pattern of development which may be quite different in different genotypes. The development of fruit size in various types of tomatoes (Houghtaling, 1935) may be cited as a simple example. In *Lycopersicum esculentum* fruit size varies from the small cherry types with a diameter of about 1·5 cm. to the standard commercial ones of 10 cm. or more. At least one major gene affecting fruit size has been demonstrated by Lindstrom (1928) and has been found to be linked with a number of qualitative genes. A study of the ovary primordium in its earliest stage in the flower buds shows that it is considerably larger in the large-fruited types than in the small-fruited ones, and that this difference is due to its possession of more cells rather than larger ones. These differences in cell number are even more pronounced in the mature fruit. Cell size, however, is also an important factor in fruit size, for there is a very close correlation between these two characters at maturity, although the range in the size of the cells is much less than in that of the fruits.

We might conclude, therefore, that differences in fruit size in tomatoes are due primarily to inherited differences in cell number and secondarily to inherited differences in cell size. A study of the actual developmental history, however, shows that the relationship in each case is a very specific one. If the volume of cells in the ovary wall (or pericarp) is plotted against volume of ovary (or fruit), both logarithmically, the curves which result for three of the fruit types are as shown in Fig. 1. The growth of the ovary to about the time of flowering involves very little increase in cell size and must therefore be due almost entirely to increase in cell number. Evidently the entire tissue is meristematic at this time. Shortly after flowering in each type, the line turns upward at an angle of about 45°, abruptly in the larger types and more gradually on the smaller ones, until maturity is attained. This shows that growth of the developing fruit after anthesis is directly proportional to the increase in size of its cells and that cell division in the pericarp tissue must have ceased (since otherwise fruit size would increase faster than cell size). Evidently in these types growth consists of two clearly distinct phases, the first of cell division and the second of cell enlargement. The essential feature of this developmental history is that in the progressively larger fruited types the actual extent of each of these phases is increased but that their relative extent is much the same, so that a similar developmental pattern, on a progressively larger scale, is produced. In other words, cell division is carried on much further in the large-fruited type than in the "Cherry," so that the ovary at anthesis is much larger; but cell enlargement is also carried on much further, so that the relative length of these two phases is similar in these two very diverse types. If cell number were the determining factor in fruit size, the length of the first phase would be related to the final size attained, but the length of the second would be constant, bringing cell volume up to a uniform, presumably optimum, level. If cell size were the determining factor, the length of the first phase would be constant and the length of the second would be related to final size. Neither of these results obtains, for *both* phases are correlated with final

fruit size. For a given absolute size of developing fruit there will be considerable variation in cell size and cell number depending on the specific developmental type to which the fruit belongs. The final size in each case seems to be the point at which a specific schedule or pattern involving both cell size and cell number is completed. When this is attained, growth stops, although there seems to be no *a priori* reason why one or both of its phases should not continue. Evidently this pattern is determined very early in the primordium of the developing organ by the genotype and then, barring environmental accidents, proceeds through its regular course. A similar result was obtained by Reed (1927) in his study of growing stems and
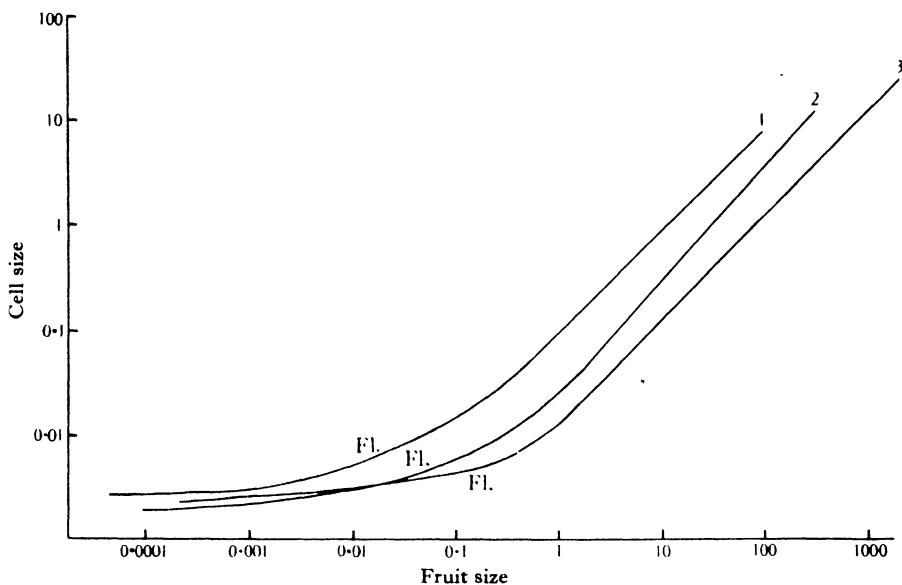
Fig. 1. The relation between average cell volume in ovary wall (and pericarp) and volume of ovary (and fruit) in three varieties of tomato, from very early floral primordium to mature fruit. Plotting is logarithmic in all cases. Unit of cell volume, 10^{-2} c.mm.; of fruit volume, 10^{-1} c.c. *Fl.* point at which flowering occurs. 1, "Cherry"; 2, "Plum"; 3, standard commercial size (variety "Bonnie Best"). (After Houghtaling, 1935.)

branches, for he found that the growth at any given period was a function of that finally attained, and that ultimate growth was therefore essentially determined at the very beginning of development. Castle and Gregory (1929) reached the same conclusion for growth in the rabbit.

Developmental studies in other plant organs indicate that the character of these growth schedules may vary considerably from species to species and from one tissue to another, but that they exist and are essentially constant in comparable material and under comparable conditions seems altogether probable. Evidence from animals on this point is as yet very meagre, although the early determination of growth patterns in the extremities of the fowl, described below, is an indication of a similar situation.

If size differences are thus related to specific developmental patterns or schedules with definite end-points, the genic differences which we believe to be responsible for size presumably have their most immediate effect upon the entire developmental programme rather than upon its various constituents. The inherited differences which have been shown to exist in cell number, cell size, and organ size at particular stages of differentiation are thus probably but the indirect results of genically controlled differences in the general schedule of development. This conception brings the problem of the genic control of quantitative characters to the same level as that of the more complex differences involving form and structure which we shall consider later.

The physiological mechanism by which those genes which determine differences in amount of growth produce their effect is still largely a matter of speculation. The increasing number of instances where it has been shown that particular metabolic or developmental rates are definitely under the control of genes, and the many other cases in which this is presumably true, suggest that differences in rates of growth play an important part in the determination of size.

On the other hand there is evidence that differences in growth rates are not always decisive in determining ultimate size. Thus a comparison of the true (logarithmic or percentage) growth rates of large-fruited and small-fruited tomatoes indicates that there is little difference between the rates under the same conditions. Ashby (1930) maintains that there is no difference in true growth rate between F_1 maize which shows hybrid vigour and grows to a great size, and the much smaller inbred parent lines. He believes that differences in embryo size, rather than in rate of physiological activity, are responsible for the conspicuous size differences at maturity.

The effects of differences in growth rate probably depend upon the specific time in development when they arise. A temporary retardation in the rate at a specific period in early development appears to be responsible for the shortness of the limbs in the Creeper fowl (Landauer, 1934) although the size of the body is but little affected, and the percentage rate of growth after the retardation is similar for both the short- and long-limbed types. One effect of the " Bar" genes in *Drosophila* is probably to change the rate of eye growth at a critical period when eye size is being determined, although the size of the body is not affected (Hersh, 1928). In the plant cases quoted the divergence in size has apparently occurred before visible differentiation has taken place, and it is not known whether, in these early stages, temporary growth differences determined the result.

One other method of arriving at some general ideas of the action of genes affecting size is to examine their interactions in development, particularly to determine whether, as has been generally supposed, the effects of the several genes concerned in a size difference cumulate arithmetically, each adding a constant quantity in any combination. If this is so and if segregation among these genes is independent and dominance lacking in the F_2 from a cross of races differing in size or other quantitative characters, the individuals should be symmetrically distributed about the mean for the trait in question. A study of such F_2 segregations in various

animals and plants shows that this is very rarely true, but that, instead, the curve for the population is skewed consistently to the left (positively) when the data are plotted in the ordinary arithmetic fashion. If they are plotted logarithmically, however, an essentially symmetrical curve results. This has recently been shown convincingly in these laboratories (Warshawsky, unpublished data) by an analysis of twenty F_2 populations segregating for weight of fruit, in *Cucurbita pepo*. Such a result should be expected if each gene, instead of having a constant additive effect, had an exponential (percentage or geometric) effect. Such exponential results are familiar in physiological processes, especially in enzyme action, and this may provide a clue as to the manner in which these genes produce their effects.

III. SIZE OF PARTS.

Organisms differ genetically not only in total size but in the relative sizes of their different parts, this being one feature which determines the form of the whole. Before we discuss the influence of genes upon form and shape as such, we may first inquire what effect they have upon the relative growth of these organs or parts.

There are many analysed cases which show that single genes influence the relative sizes of different parts apparently independently of the size of the whole organism. Thus marked differences in the relative lengths of digits and of whole limbs in vertebrates have been shown to depend chiefly on single-gene differences in the case of brachydactyly (man and fowl); the short-legged or Ancon variation in sheep and the similar "Creeper" mutation in the fowl; the length of tail in the fowl, mouse and cat; the length of the external ear in the mouse and the sheep; hair length in several cases (angora mutation of rabbit, guinea-pig, cat and others); the size of comb and head appendages in the fowl, and numerous others. Among insects one has only to look through the long list of gene mutations in *Drosophila* to find many instances of effects on wing size (apterous, the vestigial wing allelomorphs, miniature); eye size (Bar, Lobe, eyeless); leg length (ancon, dachs); bristle length (bobbed and many others). In plants there are numerous cases in which the size of a part is inherited independently of that of the entire plant. Differences in the size of flowers, fruit, seeds, leaves and hairs have been shown to be due to single-gene differences. Effects of this sort are so common as to leave little doubt that single parts are markedly affected by single genes. In many cases, of course, the same gene that modifies the size of a part may also have other effects on viability, fertility, life length and sometimes indirectly on total size, but in many others total body size is so little influenced that the final effects of the gene may appear chiefly in one or a few parts.

The existence of these cases of form differences dependent on single genes side by side with the very large number of cases in which differences in size of parts as well as in total size are inherited in a blending fashion as though dependent on many genes with small quantitative effects, has led to much discussion (Castle, Sumner, Davenport, Wright, Green) of the action of multiple size genes, particularly as to whether they are prevailingly general in their action, affecting all parts, or whether the chief effects of some are special (limited to certain parts) or group (limited to

related groups of parts such as the bones of the head or of one extremity in mammals). The most conclusive answer has been obtained by Wright (1932), who by special calculations from the data of MacDowell and of Castle on the rabbit and of Dunn on the fowl has shown that although most of the variance in bone lengths is due to factors with general effects, a minor but appreciable part must be due to those with special or group effects. Moreover, Green (1934) has given evidence from a species cross in mice which tends to show that factors with special and group effects are present and show linkage with a known colour gene.

A number of localised size differences have been analysed quantitatively in the pedicel anatomy of chromosomal mutants of *Datura*. Here the differences are related to particular chromosomes but are doubtless due to the genes which these contain. One chromosome (6 · 6) which produces the mutant "Areolate" when in trisomic condition, stimulates the development of vascular tissue and another (1 · 1) reduces it, although fundamental tissue is not affected in either case. Chromosomes 3 · 3, 10 · 10 and 23 · 24 *reduce*, while 17 · 18 *increases*, the area of the pith in proportion to that of the cortex. Still other chromosomes increase the cell size in fundamental tissue but not in vascular, while others have the opposite effect. The relative development of phloem to xylem and various other localised growth differences are also affected by particular chromosomes.

Thus the present weight of the evidence is in favour of the view that genes affecting size are for the most part general in their action but may also influence certain parts to some extent independently of the whole. In the animal, at least, this may well be due to the complex and changing nature of development in which different parts are in different stages of growth at the same time and may thus react differently to general effects.

The chief importance which this question has for our present discussion is in indicating the channels through which these genes may exercise their control. We have already shown that differences in general size may be brought about through effects of genes on the rate of growth and cell division at specific times (cell number), on the limiting sizes of the cells, and on combinations or interactions of these effects in a time schedule also under genic control which imposes a programme or pattern upon development. Inherited local size differences undoubtedly arise through genic effects on the same primary mechanisms, and it can be shown from developmental analyses of some of the local size characters cited above that the genes affecting such characters may exert a general effect on the growth rate, their special effects on separate parts being determined by the condition of the part at the time when the general modification occurs.

The general model for this type of interpretation was given by Goldschmidt in his explanation of the origin of the pattern of the butterfly's wing and later was generalized in his *Physiologische Theorie der Vererbung* (1927). The morphological facts showed that the embryonic (late pupal) wing consists of surface areas with different rates of growth. The depth of colour eventually developed in these areas, he assumed, depends upon their condition at the time when a general colour reaction occurs (Fig. 2). Areas already chitinised, having undergone rapid growth,

have passed the stage when the wing scales may become coloured; those which have developed more slowly, however, become coloured (dark). Genes (for melanism) or external conditions (low temperature) which retard development at times when certain areas have not yet passed the critical stage, increase the extent and may alter the distribution of the dark areas.
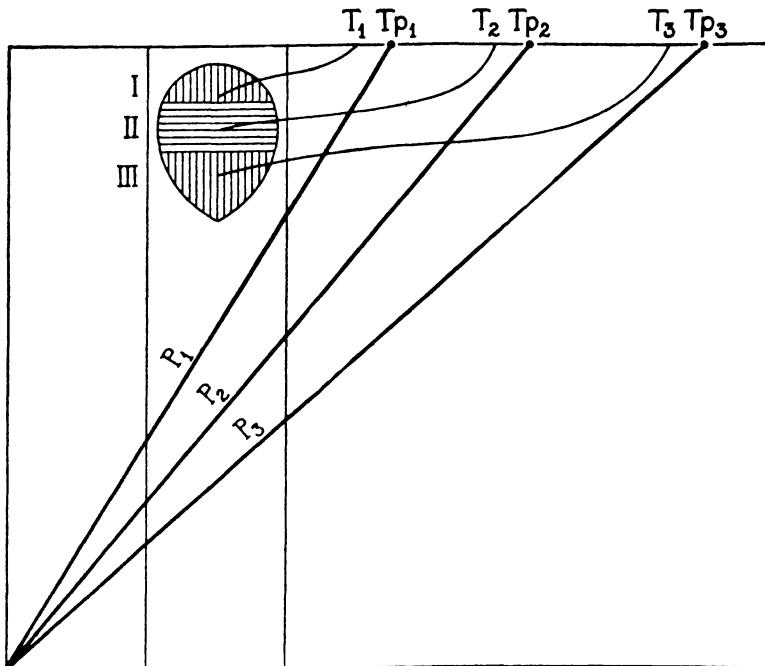
Fig. 2. Diagram showing method of development of colour pattern in the lepidopteran wing as the result of varying rates of differentiation and chemical change. I, II, and III are areas differentiating at different rates, shown by curves T_1, T_2, and T_3. The upper horizontal line represents the developmental threshold which permits of the deposition of pigments. P_1, P_2, and P_3 are three independent gene-controlled chains of reaction which are supposed to result in the formation of some component requisite for the final deposition of yellow, red, and black pigment, respectively, within the scales of the wing at times Tp_1, Tp_2, and Tp_3. At the time Tp_1 only the area I is ready to receive the stuff P_1, and therefore only this area will contain yellow scales; and areas II and III will thus become red and black, respectively. (From Goldschmidt, 1932.)

The most thoroughly analysed case of genic alteration of the sizes and proportions of parts in animals through a mechanism of this sort has been provided by Landauer's study (1931, 1934) of the development of the Creeper (short-legged) condition in fowls. Creeper fowls differ from normal ones in having much shorter limbs, the legs being more reduced than the wings as compared with the normal. Such fowls differ from the normal type by a genetic factor (either a dominant gene or a deficiency) which when heterozygous is responsible for the reduction in limb length and the altered bone structure of the Creeper. A comparison of the growth of

the skeleton of such heterozygotes with that of their normal sibs from the seventh day of embryonic life until maturity shows that the limbs of the Creeper are shorter than those of the normal from the earliest stage in which they can be measured, and that thereafter growth follows a generally similar course in Creeper and normal although minor deviations in growth intensity occur. The difference in length of limb bones has therefore arisen before the seventh day and the evidence indicates that it is a consequence of an earlier period of slower growth in the Creeper embryo. The various bones of the Creeper are not equally affected. The wing bones are less shortened than the leg bones; and in each limb the more distal long bones (those laid down later in development) are more reduced. The bones formed earliest therefore tend to escape the effect of slower growth. The longer bones of both limbs (those with greater inherent growth capacity) are the most reduced in the Creeper, indicating that the slower growth rate affects the structures in the order of their growth activity during the period of growth retardation. More-over, the minor deviations from normal growth in the Creeper bones during later development affect nearly all of them synchronously, showing that the factor has a *general* effect on growth.

These facts find their interpretation in the clear demonstration that the cha-racters of the homozygotes are due to a general retardation in growth occurring during the first three days of embryonic life. Such homozygotes die at about 72 hours of development. They can be identified just before death by their small size, absence of hind-limb buds, and by typical abnormalities in the heart, eye, and other organs. The degree to which each affected part departs from the normal corre-sponds to the order of the relative rate of growth of that part in normal development; that is, those parts which in the normal are growing most rapidly at this early period are the most affected. In the period between about 36 and 72 hours of incubation the homozygotes suffer a marked and general retardation in growth, and this is assumed to result in the apparently specific and localised abnormalities of the Creeper homozygotes. Thus the posterior limbs are most affected, since these are in the most active stage of growth during the retardation period; while the wing bud which has appeared somewhat earlier has passed the stage at which the maximum effect of the retardation can be registered.

On rare occasions a homozygous embryo survives the lethal period, although none has ever hatched. These present a most striking picture of extreme abnor-malities: great reduction of limbs, especially of legs which may be represented only by the digits and a vestige of one of the long bones; entire absence of calcification; abnormal head shape and complex abnormalities of the eye. These show the same rule in operation: the order of alteration of the structures by the Creeper genes is the order of their relative growth intensities during the earlier retardation period, and this holds good even within the parts of a single organ such as the eye. Lan-dauer's conclusion that "all observations indicate that the Creeper traits are caused by a general and unspecific retardation of body growth at a definite period of embryonic development" (1934, p. 41) appears to be sustained by the now consider-able evidence. It is supported also by the results of tissue culture experiments of

H. B. Fell (unpublished, quoted by Landauer, 1934), showing that the effects on bone growth are non-specific in nature.

The differences between the effects of one (heterozygous) and two (homozygous) Creeper factors are assumed to be due to the earlier and more severe retardation brought about by two genes and to the limitation of the effect of one gene (by its later onset) to alterations in relative size of skeletal parts and changes in histological structure, since the limb buds have probably already been formed before the later retardation occurs. The conclusion of most importance for our present purpose is that changes in the parts of the body which might appear to be effects of a gene acting locally are in this case by-products of a general effect impinging on growth and differentiation processes which are proceeding at different rates in different parts.

A condition in mice which seemed to be similar to the Creeper case has recently been studied in these laboratories by Chesley (1932, 1934). The character chiefly affected is the relative length of the tail. The short-tailed or brachyuric mutation had been shown by Dobrovolskaia-Zavadskaia and Kobozieff (1930) to be inherited as a dominant, either as a single gene or a specific deficiency, and the segregating ratios indicated as in the Creeper case that the homozygotes probably died during embryonic development. Direct examination of the embryos by Chesley showed that the short-tailed heterozygotes were indistinguishable from their normal sibs until the 12th–13th day after fertilisation, at which time a constriction appeared in the tail, followed by resorption of the portion distal to the constriction. Histological examination showed that this and other abnormalities of the vertebral column were often accompanied by local failures of the notochord to undergo normal growth and differentiation.

Examination of the homozygous embryos gave more critical evidence on the chain of events through which the genes produced their apparently local effects. These embryos were found to die regularly at the end of the 10th day of development. They were recognisable just before death by their smaller size, by the absence of tail bud and of posterior limbs, by gross abnormalities of somites and neural tube, and other features in which they differ from both their normal and heterozygous litter mates. Histological study of these homozygotes from the 8th day until death showed that both growth and differentiation are markedly affected. The notochord, although it probably begins to differentiate in early stages, fails to continue normal development and is completely absent in homozygotes before death. The neural tube becomes markedly abnormal and the evidence shows that this failure is probably a consequence of the failure of the notochord. The metameric segmentation characteristic of the vertebrate embryo is upset and normal somites are absent or reduced in number. These changes are especially marked in the posterior region and the posterior limbs and tail bud do not develop.

General growth changes affecting the size of the embryo appear to accompany rather than to precede the appearance of the specific abnormalities in the tissues and in this the case differs from that of the Creeper fowl. There are some indications that the effects of two brachy genes (or deficiencies) are exerted in very early stages,

perhaps in the primitive streak stage, but further statements about the relative specificity of the effects of the genes involved must await a detailed study of these stages. It is already apparent, however, that in this case the effects of the genes are not limited or localised in merely one or a few structures and that they are related to the time order of development of the structures involved. A major effect is registered primarily on the notochord and secondarily on the neural tube, and because of the dependence known to exist between these tissues in vertebrate development it can be said that this genetic condition has affected one of the determinative mechanisms of form development; or, in cruder terms, it has disturbed an organiser relationship.

The lethal effects of the homozygous condition may be due to disturbances of the organisation of the embryo as a whole rather than to specific effects on single tissues or parts, since Ephrussi (1933) has been able to cultivate *in vitro* some tissues of the lethal embryos far beyond the stage when the organism itself dies and to observe differentiations which never occur in the intact homozygote.

The effects of the heterozygous condition appear later and are much less severe, being limited chiefly to reduction in the number of vertebrae. These are most marked in the most distal parts of the vertebral column, those which are laid down last in development, so that this localisation may be in part due to the lateness of onset of the effect of one gene (or deficiency) at a time when the dependence relationships (as between notochord and neural tube, for example) are in a labile state only in a restricted part of the embryo.

Although the effects of the brachyury mutation appear at present to be more specific than those of the Creeper, the appearance of purely localised action of the genes is again shown to be illusory. The mere fact that both these genes are shown to exert their effects on very early stages of development when many of the complex interrelations of the vertebrate embryo are still in process of determination, should on the contrary lead one to expect general and widespread changes to result, and it is not surprising that these have lethal consequences.

In many other vertebrates, also, the size and form of the tail have been affected by hereditary changes although in most of them adequate studies of development have not been made. Rumplessness in the fowl, for example, has been shown to be due to a dominant gene mutation (Dunn and Landauer, 1934). The homozygote generally lacks the caudal vertebrae (*i.e.* is completely rumpless) and the uropygial gland, and is often sterile and less viable than the normal. The heterozygote may be rumpless or may show intermediate grades of development of the tail and rump, depending on other genes inherited separately from the rumpless gene. The tail vertebrae of the rumpless embryo are missing from very early stages (Du Toit, 1913), although the rest of the body develops apparently normally.

Rumplessness may also arise independently of the rumpless gene (Dunn, 1925) probably as the result of an accident or environmental change early in development. In such cases the condition is not inherited. Danforth (1932) has shown that variations in the incubation temperature in early development may induce this rumpless variation in normal embryos; so that it is not unlikely that here, too, a

general effect on the rate of development may produce effects limited to certain parts.

Finally, Kamenoff (1934) has studied the effects on development of a recessive gene in mice which, when homozygous, results in the condition known as kinky or flexed tail. The tails of such mice show sharp angles and stiff joints and are sometimes also shorter than normal. This is due to fusions, usually unilateral, between neighbouring vertebrae. These originate during the 14th day of embryonic development in a lowered rate of growth (usually unilateral) in some of the intervertebral cartilages and the subsequent failure of such areas to differentiate into the normal felted fibres which form the intervertebral discs separating the vertebrae. Cartilage which does not so differentiate becomes ossified and forms bony bridges or fusions between vertebrae. These occur most frequently in the tail but may also occur with diminishing frequency in the more anterior portions of the vertebral column. The gene also affects the composition of the blood of the embryo. The red cell count of the flexed embryos is definitely below that of the normal from the 13th embryonic day (when first measurements were made). This anaemia persists through embryonic development but disappears during the first few weeks after birth. In addition the general growth of the flexed embryos is somewhat retarded in late stages, possibly as a result of the anaemia, and there is a persistence of certain embryonic blood cell types (Mixter and Hunt, 1933). Kamenoff has assumed that a general retardation of growth has occurred in the flexed embryos at a critical period in the development of the intervertebral discs, but direct evidence of this is not available. It is not unlikely that such an effect may result from the reduction in the oxygenation capacity of the blood, but changes in both cartilage and blood may be common results of antecedent and at present unknown changes in development brought about by the flexed gene.

In addition to the recent studies described above there is the now well-known analysis by Goldschmidt (1927) of the effects on development of different conditions of balance of the sex genes in *Lymantria*, which showed that the degree to which the sexual structures were affected by various dosages or excess "strengths" of the genes of one sex over those of the other, corresponded to the *time order* in which the structures appeared in normal development. Two stable points of balance of the sex genes were assumed, one in which the "male" genes or their products were in effective excess from the beginning, leading to early differentiation of indifferent structures into male structures, the other in which "female" genes were in effective excess leading similarly to female structures. Where the excess of one type of gene effect fell below a minimum quantity (the epistatic minimum), early development was controlled by the genes then in excess; but after a critical period or "turning point" in development, the position of which was determined by the relative excess of one type of gene effect over the other, structures not yet determined showed characteristics of those of the opposite sex. The evidence rests upon a genetic analysis of the inheritance of intersexuality, and upon a morphological and developmental study of the sexual organs of normal male and female and of intersexes of various degrees. It is clear that in a low-grade intersex (one with only slight

traces of structures of the opposite sex) the structures altered are those which appear latest in normal development. As higher and higher grades of intersexuality (those more and more modified toward the opposite sex, ending in complete sex reversal) are approached, the structures modified are those which appear earlier and earlier in development. The different allelomorphic conditions of the sex genes may, according to Goldschmidt, produce localised or more general effects according to the period in development at which they become effective, and this in turn depends upon the relative quantities of the two genes involved and the relative speeds of reaction which they determine in proportion to their quantity, the greater the relative quantity of the gene, the more rapidly (and hence the earlier in development) is its effect produced. The effects on development of the different degrees of balance of the sex genes in *Drosophila* apparently operate through similar mechanisms (Dobzhansky and Bridges, 1928).

We may turn now to a few cases in which the development of genic variations in the relative sizes of parts has been studied by other than strictly morphological methods. In *Drosophila*, for example, it has been possible to investigate the effects of certain genes by modifying the environment at critical periods during larval development. In the mutant type known as Bar the compound eye is smaller and narrower than the normal, and the gene responsible behaves as an incomplete dominant to the normal. Zeleny and his students have carried out a concerted attack on the problem of how the Bar gene and its allelomorphs affect development. Seyster (1919) showed that the number of facets in the Bar eye (in the case of Bar and its allelomorph Ultrabar) varies inversely with the temperature at which one period of early larval development is passed. This has been called the temperature-effective period (Driver, 1926) and corresponds to a period during which the form and size of the compound eye are determined. (This period, as Speicher (1934) has shown in the case of the eyeless mutant in *Habrobracon*, coincides with the period of most rapid growth of the tissues affected.) By modifying the rate of development at this time an apparently specific effect on the eye is brought about, but only if the Bar gene or one of its allelomorphs is present; temperature has but little effect on the normal. The Bar genes thus alter the reactions occurring at this time, making them more labile and responsive to external changes. They also influence, according to Driver's (1931) evidence, the position of the period in time and its duration. Hersh (1928) has shown that the two lobes of the eye react to temperature with different logarithmic growth rates and suggests that the Bar genes act by altering the distribution of growth in early development. He thinks that the critical period may have different relations to the general growth of the zygote according to the Bar allelomorph which is present. These genes, therefore, may be assumed to affect the relationship between the growth intensities of a part and the whole, the localisation of the effect depending upon the conditions obtaining during some critical period.

From the descriptions of the several cases in which the effects of genes have been studied during development, it is clear that those affecting form and size act generally in early stages (in animal development) and often can be shown to affect

the growth of the parts relative to each other or to the whole. The sizes and forms eventually attained appear not to represent mere continuations or extensions of the later stages of a growth period, but to arise from alterations in the relations of the parts occurring during early determinative periods.

The relationship between the order of events in development and the relative effects of the genes on the various parts which is assumed in Goldschmidt's theory is clearly borne out in several of the cases discussed above. The earlier onset and greater effects of homozygous than of heterozygous genes, while not conclusively proved, is made highly probable, and so far as it goes points in the direction of the speeds of reactions being proportional to the number of genes present. Finally, the evidence from a morphological examination of different structures during development shows that local effects on the sizes and forms of the parts may be brought about by genic influences on processes of a more general character. A part of the effects, at least, appear to follow from the modifications imposed by mutant genes upon a fundamental pattern of organisation in which there are inherent differences in the reactivity of the parts. The latter may be assumed to be due to the hereditary constitution of the species or group of which the pattern is typical, but the effects of single genes upon this deeper organisation are not sufficiently known, perhaps because wide departures from it result in death in the earliest stages. The effects described above are thus of a secondary order as compared with those which impose this organisation.

Conclusions drawn from a study of animals, with their closely integrated growth, often seem inapplicable to plants, for here the possession of a very loosely organised body and the generally indeterminate character of growth makes the relationship of the part to the whole rather different from what it is in animals. In comparison with the animal, the plant shows so little integration as to constitute in many cases no more than a quasi-colonial entity. In such an "individual" there might well be almost complete independence between organ and body, and the evidence shows in many cases that this is so. The sizes of leaves, flowers, fruits, or other multiple organs, especially in such large plants as trees, bear little evident relation to the size of the whole organism. Sinnott (1921) has shown that in young or small bean plants there is close correlation between organ size (leaf or fruit) and plant size, both increasing together up to a certain maximum point, but beyond this the further growth of the plant, which may be considerable, is due merely to a multiplication of similar-sized parts. Here it is probably the size of the growing point, increasing to a maximum diameter and then remaining constant, which is related to the size of the organs it produces. Growth correlations comparable to those in the whole animal body are found only in one of these masses of meristematic tissue and the structures directly produced by it.

Within single determinate plant organs, however, growth interdependencies are as evident as in animals. In *Cucurbita*, for example, both fruit size and seed size • are clearly inherited, and there may appear to be a certain amount of independent hereditary control of these two traits; but it is also true, particularly in smaller fruits, that seed size is closely related, developmentally, to fruit size. It is obviously

impossible for the large seeds of the "pumpkin" type to be produced by the smaller fruits of the gourd. What is inherited here is therefore evidently a particular relationship between fruit size and seed size.

IV. GENIC CONTROL OF SHAPE.

The chief effect of the genes which have been discussed in the previous section is apparently to control specific growth relationships between the various parts and between a part and the whole. Huxley (1932) has studied extensively the relationships between the growth of an organ and that of the whole body, and has used Pézard's term *heterogonic growth* to describe those cases in which a part is growing at a different rate from the whole. Huxley finds that in such cases the *relative growth* of one part to another or of part to whole, when true or logarithmic rates are compared, is surprisingly constant[1]. This indicates that a factor which changes the rate of enlargement of a part does not have a purely local effect but that the growth of the part is integrated with the growth of the entire organ or organism. The constancy of these relative growth rates has now been demonstrated in so many specific instances and so many aspects of both plant and animal development as to lead to the belief that growth is everywhere a correlative process and that genes which modify it locally do so only by modifying the character of a basic growth pattern for the entire organism.

Although the genic control of heterogony can well be investigated in such cases of local growth differences between parts and whole as have been analysed above, the genetic basis of relative growth in its most simple aspect can best be approached by a study of the dimensional relationships within a single organ or part. Since such dimensions ordinarily grow at rates bearing constant relations to each other, characteristic organic *shapes* result. A knowledge of the inheritance of specific shapes or forms will therefore provide important information as to the role of genes in controlling these simplest of growth relationships. The inheritance of shape differences has now been studied quantitatively in a number of organisms with results of interest for our general problem.

The specific problems to be considered are whether differences in the shape of a part are directly determined by genes, and how such determination, if it exists, is effected.

There is ample evidence from both plants and animals that differences in the shape of the body, organs, and other parts are inherited. Leake (1911), Hutchinson (1934) and others have shown that leaf shape in cotton may be analysed in simple Mendelian terms. Shull (1914) showed that the difference between triangular and top-shaped fruits in *Bursa* depends on the action of two duplicate genes. Imai (1930) has found a long series of genes which control leaf shape in *Pharbitis*. Many floral differences, such as peloria, have been shown to be dependent upon single ·genes.

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[1] The ratio between the rate of growth of a part and that of the whole (or of one dimension to another) may be expressed by the value of a constant exponent, k, in the equation $y = bx^k$, where x represents the size of the whole, y of the part, and b the value of y when $x = 1$ (Huxley, 1932). When both are growing at the same rate, the value of k is obviously 1.

In animals, the form of comb in poultry was one of the first shape characters to be analysed on a Mendelian basis (Bateson and Punnett, 1911), and has long served as a classic example of genic interaction. Many differences in wing shape in *Drosophila* can be assigned to a single-gene difference from the wild type. A gene has recently been described which makes the body of the larva shorter and broader than normal but is without effect on the body of the adult. Numerous other instances could be cited.

There has been less success in analysing the pronounced differences in general form and conformation which characterise the various breeds and races of domestic and laboratory animals. Dunn (1928) has shown that small differences in head shape and in the proportions between various bones of the body of the fowl come to distinguish various families descended from the same stock by close inbreeding, indicating clear hereditary differences. Variations in head shape and body build in man, in spite of the attention paid to them by anthropologists, have received no clear interpretation, although many of them are undoubtedly inherited.

In addition to the instances of shape inheritance cited above (and the list is very far from complete) a few recent cases have been subjected to both Mendelian analysis and developmental study. The data are most complete for fruit shape differences in several plant genera, especially *Cucurbita*. Several genes affecting fruit shape have been identified in the summer squash, *C. pepo* (Sinnott, 1927; Sinnott and Hammond, 1930). The difference between the flattened disc or "scallop" type (equatorial diameter much greater than polar) and an essentially spherical one is evidently due to a single gene, since there is a clear monofactorial segregation into the dominant disc and recessive sphere types in F_2. The difference between the disc and another spherical type, somewhat more elongate, is due to another gene, which also segregates sharply. If these two spherical types are crossed, the disc fruit shape is reconstituted, indicating that it is the result of an interaction between the dominant allelomorphs of these two genes. The F_2 from such a cross gives 9/16 discs (the double dominant), 6/16 spheres (of two types), and 1/16 of a new elongate type (the double recessive). Evidently both "sphere" genes have a flattening effect on fruit shape and this is cumulative when they are brought together, resulting in the disc form. These four shape types are thus determined very simply as the result of the activity of two independent pairs of genes. Other genes affecting fruit shape have also been identified in this species which either inhibit the flattening effect or may themselves elongate fruit shape. There is good evidence that this single character, fruit shape, is the result of a rather complex balance between an extensive series of genes, differing among themselves in the nature and intensity of their effects.

Genes with similar effects have been found in *Lycopersicum* (Lindstrom, 1928), where the difference between the elongate type and the spherical one is clearly due to a single gene. Similarly in *Capsicum* (Kaiser, unpublished data) a single-gene difference has been demonstrated between isodiametric and elongate types.

It may be objected in such cases that even though there are monofactorial ratios for shape, it is not shape which is directly affected by genes, but rather dimensions,

volume, or some other purely quantitative trait; and that shape is merely a resultant of these more immediate genic effects. In the case of the fruits of *Cucurbita pepo*, Sinnott (1935) has shown that shape is inherited quite independently of volume or of particular dimensions, and that there are presumably genes which control it directly. The lines of evidence on which this conclusion is based are as follows. (1) Such simple, clearly segregating shape differences as have just been described in this species are inherited independently of size. If a heavy disc type is crossed with a light spherical one, the segregating disc and sphere fruits in the F_2 are equal in weight and intermediate in this regard between the parental types. (2) In the F_2 from crosses between types differing in both shape and weight, there is rarely any significant correlation between these two traits. (3) In the F_2 from crosses between types differing in shape, the segregation for shape index is much sharper than for any of the dimensions alone. (4) In pure lines and F_1 populations, which are thus homogeneous genetically, there is a *positive* correlation between fruit length and fruit width, the variability all being due to environmental factors. In every F_2, however, from crosses between types differing in shape there is a *negative* correlation between length and width. This is what must occur if shape is determined independently of size, since every elongation must then result in reduction in width, and *vice versa*. (5) In pure types and F_1 populations there is no difference in the variability of fruit length and width, but in the F_2 from crosses between types differing in shape, and thus segregating for this character, length is approximately *twice* as variable as width. This is to be expected if volume and shape are independent, since in a radially symmetrical organ like the fruit, a given change in width will evoke a compensatory change in length of twice its relative magnitude, when volume is constant.

If, then, differences in fruit shape are determined by genes directly, how do these differences arise in development? Comparative study shows that the method is not the same in all cases, though it always consists in the control of relative dimensional growth. In the fruits of the pepper, *Capsicum* (Sinnott and Kaiser, 1934), the young ovary up to about the time of flowering is essentially isodiametric and is similar even in types which are widely different in mature fruit shape. In the spherical-fruited type this shape persists as the ovary ripens into the fruit, the constant of relative growth remaining approximately at 1. In the elongate-fruited ones, however, shortly after flowering length begins suddenly to grow at a much greater relative rate than width, so that as the fruit enlarges it becomes more and more elongate in shape index. Between two such types there may be only a single-gene difference, and this evidently operates to change the relative growth rate of length and width at a particular point rather late in development. The entire effect of the gene in the elongate types is more complex than this, for the primary difference is associated with a definite gradient along the main axis, as is shown by the progressive decrease in width and in number of seeds from the base to the apex of the fruit.

In the tomato, *Lycopersicum*, on the other hand, Houghtaling (1935) has found that the difference between the pear-shaped and the spherical fruit types is esta-

blished before flowering rather than after. Here in the early primordium length grows faster than width in the elongate race, but the two are approximately equal in rate in the sphere, with the result that a rapid divergence in shape occurs very early. After flowering the relative growth rates of the two dimensions are approximately equal in both elongate and sphere, so that the differences thus early established are maintained through later growth with no further divergence. The developmental origin of fruit-shape differences is therefore quite unlike in time in these two genera of the Solanaceae.

A third developmental type is presented by the squash, *Cucurbita* (Sinnott and Kaiser, 1934). Here the fruit-shape differences, which are more extreme than in either of the other genera described, are apparently already established in the very earliest primordia. When the primordium is considerably less than a cubic millimetre in volume, the shape to which it will develop in the fruit is already evident. In all types the relative growth rate of the dimensions throughout development is almost the same, being consistently a little higher for width than for length, so that there is no visible divergence whatever. Evidently the shape difference in *Cucurbita* is determined by the size and thickness of the tiny meristematic disc which is to produce the floral primordium. The period of visible divergence, which is of some duration in the tomato and continues throughout the entire later development of the fruit in the pepper, has been very greatly condensed in the squash.

Shape differences cannot well be expressed by dimensional indices, since these will change radically during development if the growth of the dimensions is unequal. They should rather be designated by the values of the relative growth constants (the values of k in Huxley's equation) at different stages of development. Not only the relations of length and width are involved but all the dimensions which make up a complex pattern. Thus the two "sphere" genes in the squash mentioned above produce fruits in which the length-width index is not very different, but the actual shapes are quite diverse, one type being broadly pear-shaped and the other having a high-shouldered acorn-like outline. It is such an entire pattern which is inherited, rather than a simple index.

In all these cases, then, as in the establishment of size differences, the genes appear to control entire developmental patterns or schedules and not merely particular parts or processes. Development is definite and orderly; even where its character changes suddenly, this change is part of a programme which from its earliest stages is determined by the genetic constitution. Even in the complex growth processes of the animal body where many parts are growing at different and constantly changing rates, there are precise growth relationships which form a specific developmental pattern. We have for the animal no complete analysis of the ways in which genes control the forms of organs which are comparable to the simpler instances from plants, but there are indications from changes in the proportions of the parts of the heart and of the head and of ratios between the lengths of different bones and between the length and width of the same bone in the fowl (Landauer, 1934) that these, too, arise by changes in the relative growth constants brought about by genic action.

As to the ultimate mechanisms by which genes determine shape, no final conclusions can be reached as yet. Three possible ways suggest themselves. They may operate (1) by control of relative rates of cell division in different regions of body or organ, (2) by control of cell shape, or (3) by control of the plane of cell division and thus presumably of cell polarity. The first is operative in cases of typical heterogony. The second seems rarely to be of importance, though in some plant organs there are definite correlations between cell shape and organ shape (Sinnott, 1930). It is therefore probably through a control of cell polarity, however this is attained, that genes in most cases produce their effects upon shapes.

In no case, evidently, does any single "shape" gene completely determine an organic form. It obviously acts as one component among others, both genetic and environmental, which operate during development. A specific shape is the resultant of the balanced interaction of a specific set of such factors, and only in cases where all other controlling influences remain constant will a "shape" gene constitute a decisive differential and thus reveal itself in simple Mendelian segregation.

From our present fragmentary knowledge of the genetic basis of size and form there emerge a number of general ideas which may serve as points of departure for further study. Important among these are the concepts of the interaction of genes in development, with the consequent attainment of genic balance or equilibrium, and of the correlation of growth rates into a specific developmental schedule or pattern. A brief discussion of these two ideas may be useful as an interpretation of the facts which have here been presented.

V. GENIC BALANCE.

In a discussion of the effects of genes upon characters of size and form (as well as all others) it is impossible to specify the effects of any single gene separately from the system of which it forms a part. Genes cannot act alone in development. This conclusion was first reached from observations of adult characters only, supplemented by data from genetics and cytology. It is now embodied in the familiar concept of Genic Balance, which assumes that the final characters are the results of interaction among all the genes. An hereditary change in a character is assumed to follow upon a change in the relative effects of the genes, some of which tend to influence it in one direction and some in another, the character itself representing the point of equilibrium between these diverse effects. Where the new character shows a single-gene difference from the old, it is assumed that a single gene has mutated and has thus altered the equilibrium. Single genes, according to this theory, act only as differentials, never independently of the whole gene complement. This idea, clearly, is the opposite of that which obtained during the period when genetics consisted chiefly of Mendelian analysis, for then segregation in transmission so impressed geneticists that they tended to carry this idea of the separateness and independence of genes over into development, neglecting the obvious fact that after the organism had been dissolved into such a mosaic it must somehow be put together into an integrated whole.

Although numerous instances of gene interaction and of the collaboration of multiple genes in affecting quantitative characters had long been known, the idea of genic balance was first applied to more or less continuous or intergrading changes in single traits, which could not be analysed as due to single-gene changes. The idea of characters as the resultants of reactions set in motion by genes with different or opposite tendencies was introduced by Goldschmidt to explain the intersexes of *Lymantria*, but the most convincing demonstrations of balance among the genes were provided by the combination of cytological and genetical methods employed by Blakeslee and Belling for *Datura* and by Bridges for *Drosophila*. Capsule shape in *Datura* was found to be affected not by a single gene or even a small number of them, but by genes in every one of the twelve chromosomes. Each primary mutant (a plant in which one of the chromosomes is present in trisomic condition) has a specific capsule shape, in which the effects of the chromosome which is in excess are especially stressed (Blakeslee, 1921). The effects of some chromosomes on capsule shape are in one direction and of others in an opposite one, so that this character in the normal diploid type is the result of a balance or equilibrium between the action of many genes with diverse tendencies, distributed through all the chromosomes. This phenomenon of balance has been demonstrated in many external traits in *Datura* but is especially well shown in anatomical characters, where it has been subjected to quantitative analysis (Sinnott, Houghtaling and Blakeslee, 1934). Characters of cell size, cell number, and tissue development in the floral axis on the day of flowering were measured in the diploid and in the twelve primary mutants and significant differences were found between them. The effect of certain chromosomes, for example, is to decrease the area of the pith and of others to increase it, and the value for the diploid itself is very close to the mean of the values of the twelve primary mutants.

In *Drosophila*, Bridges has shown that sex is the result of a balance between genes in all of the chromosomes. The sex-chromosome is assumed to contain an excess of female tendency genes, the autosomes to be prevailingly male; and the *relative* proportions of these genes with opposite effects determine the degree of development of the two types of sexual characters, as shown by the adult appearance of polyploid and heteroploid types with various ratios of sex-chromosomes or fragments to autosomes. Here also, a single-gene change in one of the autosomes may act as a differential, for Sturtevant (1920) working with *D. simulans* and Lebedeff (1934) with *D. virilis* have each found a mutant gene for intersexuality.

We have cited other instances of balance between a series of genes with opposite tendencies in the determination of fruit shape in *Cucurbita*, and the numerous similar examples in the literature of genetics show that the conception is of general usefulness.

VI. CORRELATED GROWTH RATES.

The possible physiological mechanisms by which genes may affect development are numerous and varied. Actually, however, we have as yet good evidence of very few. Goldschmidt (1927) and later Huxley and others have emphasised the important roles which genes play in controlling the *rates* of developmental processes,

and thus presumably of metabolic and ultimately of physical and chemical changes. This conception has been successfully applied to the interpretation of the sexual states of *Lymantria* and to various patterns and other characters in Lepidoptera (Goldschmidt, 1927), to the rate of eye darkening in *Gammarus* (Ford and Huxley, 1929), and in other cases. By assuming genic control of such rates, particularly with the further assumption of critical or sensitive periods in development which is justified by the evidence of experimental embryology, it is possible to explain almost any difference brought about by genes. Certainly a control of the rate of cell division and the rate of cell enlargement must be essential parts of the genic control over such growth processes as we have described.

Control over rates alone, however, cannot explain the co-ordination of growth processes and the constant and specific relationships between various growth rates which is characteristic of development. A given process of growth, such as cell division, does not proceed at the same rate throughout but is sometimes rapid, sometimes slower, and at certain points and times ceases altogether. Moreover, the rate of one process at any time is intimately related to the rates of others and to the conditions obtaining in the whole organism. It is through a control of these *relative rates* that the genes must exercise their effects. By this means they produce the orderly series of changes during development which we have called the developmental pattern.

Comparative embryologists have emphasised the integrated nature of individual development and the differences that exist among animal types not only in adult form but in the developmental steps by which these arise. The organisms compared, however, have been so widely separated taxonomically that it has been impossible to determine what part the genes play in these developmental differences. By comparing forms known to differ by one or at most a few genes, it is possible to show that the genic differences affect the entire course of development and alter the whole growth pattern. The differences studied are, to be sure, not very great, but they are specific and constant. Even genic differences in size are not brought about by mere extensions of growth to progressively higher limits, but by alterations of the complex schedule of growth from the very beginning.

The simplest examples of such control of relative growth rates are provided by the cases already described where a difference in shape of a single plant organ is determined by a single-gene difference. In animal development, where the processes are more numerous and complex, the effect of such a change will depend upon the particular point in this complex series at which it makes itself felt. The time factor is of so much greater significance in animals than in plants as to constitute almost a new variable which disproportionately magnifies the complexities of development. Plants, by their ability to repeat in the meristematic tissues many of the steps of development which in animals are passed through but once, tend to escape from the domination of a time sequence. In all organisms, however, the existence of a specific sequence of correlated changes in growth rates, under genic control throughout, constitutes one of the most important problems in developmental genetics.

Both of these central theories—the existence of a genic balance as a part of which each gene acts upon the whole, and of a definite developmental schedule of correlated growth rates which the genes control—are made highly probable by the evidence now at hand. To serve as the foundation for a more complete understanding of the genic control of traits of size and form, however, they must be stated in greater detail and based on a much wider body of evidence as to the actual differences, quantitatively expressed, in cells, tissues, and organs, throughout development, which are produced by known differences in gene aggregates. The accumulation of such evidence is the chief present task of developmental genetics.

VII. SUMMARY.

1. Genetics has concerned itself almost entirely with an analysis of the hereditary constitution of organisms in terms of genes. For a complete understanding of hereditary differences, however, it is important to determine how genes control the processes of development so that specific characters are produced. Traits of size and form lend themselves most readily to studies of this sort, and in a few instances direct evidence of changes in development brought about by specific genes has been obtained from both animals and plants. This evidence is briefly reviewed.

2. The influence of genes in determining size has been clearly demonstrated. Instances are described in which these genes produce their effects by a control of cell size and of cell number, and also, as is perhaps more generally the case, by determining in early stages a developmental schedule in which these two elements of growth are co-ordinated in a specific fashion.

3. The size of parts seems often to be genically determined independently of body size, but in the few cases which have been critically analysed local size differences are found to be due to general effects upon growth occurring at such a stage of development that a particular part is more affected than the rest of the organism.

4. Characters of shape and form, either of the body as a whole or of its parts, have been shown in a number of cases to be determined by genes, which evidently operate through a control of the relative rates of growth in specific directions or dimensions.

5. The underlying mechanisms by which genic control of the development of size and form is brought about are as yet unknown. In a search for them, however, it is important to recognise two general concepts which developmental genetics has established: (1) Genic balance, or the dependence of a given character upon the interaction of all the genes rather than upon a single one, each gene producing its effect not alone but by virtue of its activity as a part of the whole genic complement. (2) Correlation of growth rates in development, by which the rates of specific processes, known in many cases to be genically controlled, are so interrelated in a definite schedule or pattern that development becomes a unified and orderly process.

6. The necessity is pointed out of obtaining a much broader basis of the facts of development in genetically analysed material before generalisations of importance can be made.

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RECENT CONTRIBUTIONS TO OUR KNOWLEDGE OF THE AQUATIC PHYCOMYCETES

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CONTENTS.

I. INTRODUCTION.

AFTER a period of relative inactivity, research in the aquatic Phycomycetes ("water moulds") has exhibited during the past decade a remarkable recrudescence. This appears to have come about not only by reason of the success of a few stimulating teachers in transmitting to their students their own enthusiasm for these peculiar and obscure organisms, but also by the awakened interest of mycologists and botanists in general to the significance of the lower fungi in interpreting biological phenomena and relationships in other more highly organised groups.

In other fields, certain periods of more intense, enthusiastic, and productive work can be detected. So in aquatic mycology the few excellent papers and the monograph of von Minden (1915) might be said to terminate the first phase of research activity on these fungi, a time (1855–1915) characterised chiefly by the classic investigations of Braun, Pringsheim, de Bary, Nowakowski, Zopf, Cornu, Dangeard, Klebs, de Wildeman, Thaxter, A. Fischer, Henning Petersen, E. J. Butler, and others.

The papers of Scherffel (1925–31) on the chytridiaceous fungi and Coker's (1923) monograph of saprolegniaceous forms might well be considered to usher in the present period of renewed activity and interest, in which investigation has largely been concerned with a determination of just what forms are present in nature and an understanding of their morphology, life cycles, and relationships. In this relatively "Linnean" state of our knowledge it is inevitable that little work of significance along lines of modern physiological investigation has been accomplished, although many of these organisms because of their extensive, one-celled thallus would lend themselves admirably to it (see however, Emoto, 1923; Lilienstern, 1927; Lounsbury, 1930). Nor has much been added concerning the cytology of the group (Smith, 1923; Guilliermond, 1922; Patterson, 1927a, b; Cooper, 1929a, b; Mäckel, 1928; Carlson, 1929; Cotner, 1930a, b; Laibach, 1927; Behrens, 1931). Indeed, among the aquatic chytrids, it is not known in many instances whether the mature thallus is uni- or multinucleate. Nor are these fundamental gaps in our knowledge surprising when one considers the infrequency with which most of these fungi are found. Perhaps nowhere has the lack of knowledge of the methods of collection contributed more to the inevitable fable of rarity than in the aquatic fungi, particularly in the Chytridiales. While it is no doubt true that certain forms occur relatively infrequently in nature, yet the greater number can be collected with the application of the proper technique. For example, the highly interesting group of the Monoblepharidales has for many years been regarded as among the rarest of the fungi. As a result of recent work (Laibach, 1927; Sparrow, 1933b) a better understanding of the environmental conditions necessary for their appearance has been made known generally (see p. 171).

The Phycomycetes are ordinarily considered the most primitive of the true fungi, and as a group include a wide diversity of forms, some with definite flagellate tendencies, others closely resembling colourless algae, and still others which are true "moulds." The vegetative body (thallus) may be plasmodial (Fig. 1D), rhizoidal (Fig. 1C) and of limited extent, or be mycelial and very extensive. In any case, its outstanding characteristic is a tendency to be non-septate and in most groups multinucleate, cross-walls being laid down in vigorously growing material only to delimit the reproductive organs. The unit of non-sexual reproduction, the spore, is borne in a sporangium, and in the aquatic and semi-aquatic orders is provided either with a single posterior cilium or with two laterally attached or terminally attached ones. Sexual reproduction is accomplished in one great group (Zygomycetes) by the well-known process of conjugation of the tips of two mycelial branches, resulting in the formation of a thick-walled zygospore; only non-motile spores are formed. In the other orders, generally spoken of collectively as the "Oomycetes" (to which the forms herein considered belong), there is great diversity in the method of sexual reproduction, and as in the green algae all gradations from isogamous-planogametic to oogamous-aplanogametic types occur. Nor is the type of sexual reproduction necessarily linked with degree of thallus development. Thus, in the endobiotic, holocarpic genus *Olpidium* (Fig. 1D), a form with a plasmodial thallus, isogamous planogametes are found (Fig. 2A), while in *Allomyces*, a

eucarpic form with a well-developed mycelium, planogametes (anisogamous) are also found. On the other hand, in *Sapromyces*, a saprolegniaceous form, while the thallus closely resembles that of *Allomyces*, sexual reproduction is oogamy of a high type and involves, as in the better known genera *Saprolegnia* and *Achlya*, the formation of an oosphere contained in an oogonium, and of an antheridium; fertilisation is accomplished by the transference of antheridial material into the oogonium through a tube formed by the male structure. From the fertilised egg a single thick-walled oospore is formed. This type of sexual reproduction is characteristic of the higher aquatic and semi-aquatic orders. Others occur but these are sufficient to illustrate the diversity found in the Oomycetes.

The Phycomycetes comprise the following orders:

Zoosporic, aquatic series	(1) Chytridiales
	(2) Blastocladiales
	(3) Monoblepharidales
	(4) Saprolegniales
	(5) Leptomitales
	(6) Ancylistales
	(7) Pythiales
Conidial, terrestrial series	(8) Peronosporales
	(9) Entomopthorales
	(10) Mucorales

The Plasmodiophorales (Cook, 1933) are not regarded as members of the Phycomycetes, but appear to be more nearly allied to the Myxomycetes (see, however, Fitzpatrick, 1930).

The aquatic Phycomycetes as considered in this article comprise those members of the group which are found on various plant and animal substrata in the water or in the soil and comprise the first seven of the aforementioned orders. No attempt has been made to include the parasites of higher plants nor more than to mention the Pythiales (see Matthews, 1931), although it should be remarked that in recent years it has been found that nearly all of these orders possess members which prey upon plants of economic importance (Bensaude, 1923; Drechsler, 1925, 1927, 1928*a*, *b*, 1929, 1930; Jones and Drechsler, 1925; van der Meer, 1926; Guyot, 1927; Buisman, 1927; Kendrick, 1927; Meurs, 1928, 1934; Hemmi and Abe, 1928; Schwartz and Cook, 1928; Sideris, 1929, 1931; Vanterpool and Ledingham, 1930; Nagai, 1931; Vanterpool and Truscott, 1932; Ito and Nagai, 1932; Ito and Tokunaga, 1933; Sampson, 1932; Truscott, 1933, etc.).

II. CHYTRIDIALES.

(1) OCCURRENCE AND CULTIVATION.

The members of this order, most of which are parasitic on algae, other aquatic fungi, or flowering plants, are the most baffling and least understood of the Phycomycetes. This appears to be due not only to their apparent rarity and small size,

but also to their sporadic occurrence and transient nature, and above all to our inability to maintain them in artificial or even gross cultures. While the conditions under which a chytridiacean epidemic may occur among algae, for example, may be partially understood, no methods have as yet been devised whereby this infestation, which is usually of a few days' duration, can be maintained. Thus Karling (1931a), using a ubiquitous species of *Entophlyctis* found in filaments of *Cladophora*, has attempted to simulate optimum conditions for the maintenance of the fungus, but was unable to perpetuate it in gross culture even when new material of the dead or dying alga, some of it thermally or mechanically injured, was introduced. As Karling has suggested, variations in the degree of susceptibility of the host and virulence of the fungus are probably quite as important as immediate environmental conditions and indeed, one might add, may be governed to a degree by the latter.

Attempts to grow chytrids on artificial media are recorded in only a few instances. Sparrow (1931c) succeeded in cultivating for a time on maize meal agar a species of *Cladochytrium* found by him parasitising several genera of green algae. The most extensive work of this nature has been reported by J. Bayley Butler and Humphries (1932), who have succeeded in growing in artificial culture *Catenaria anguillulae* Sorok., a parasite of the ova of the common liver fluke of sheep and various microscopic invertebrates. In addition to maintaining it in gross cultures of living or boiled liver fluke eggs in tap water, they find that it will grow on the following media:

(1) Agar (0·25 per cent.) and fluke ova extract (derived from crushed ova, the extract being subsequently filtered and boiled).

(2) Agar (0·25 per cent.) + above extract + flaked coagulated egg albumin in equal parts.

(3) The same as preceding but without agar.

(4) Agar (0·25 per cent.) + fluke ova extract (derived from *dried* ground ova).

Solution (4), in which, by reason of drying, the most complete maceration of the eggs was accomplished, yielded a very concentrated solution which not only afforded already established thalli an excellent medium for growth but also one in which the zoospores germinated and formed sporangia. In view of Sawyer's (1929) experience in cultivating species of entomopthorous fungi on artificial media, it would not be unlikely that *Catenaria* after a period of growth on the above decoctions might be induced to grow on the ordinary types of laboratory media. Further investigations in culturing chytridiaceous fungi should be productive of interesting results, although too much weight should not be attached to the morphological diversity found under these highly artificial and special conditions of growth. The sense of security derived from the realisation that a particular chytrid will actually be available when needed should furnish a strong incentive for enthusiastic work of this nature.

(2) NEW METHODS OF DEVELOPMENT.

The greatest recent advances in the study of the Chytridiales have been along lines of the determination of what forms are present in nature and there is a growing realisation that there probably exists an enormous aquatic flora with only a few

scattered members of which we are now familiar (Scherffel, 1926a; Sparrow, 1933a).

Before considering the new types of development which have been encountered in recent years, a recapitulation of certain of the older well-established types might be of value. Four principal methods of development may, for the purposes of this article, be considered, to each of which the genus name of the fungus most nearly approximating it might appropriately be applied, viz. (1) *Chytridium*-type, (2) *Rhizidium*-type, (3) *Entophlyctis*-type, and (4) *Olpidium*-type. In the *Chytridium*-
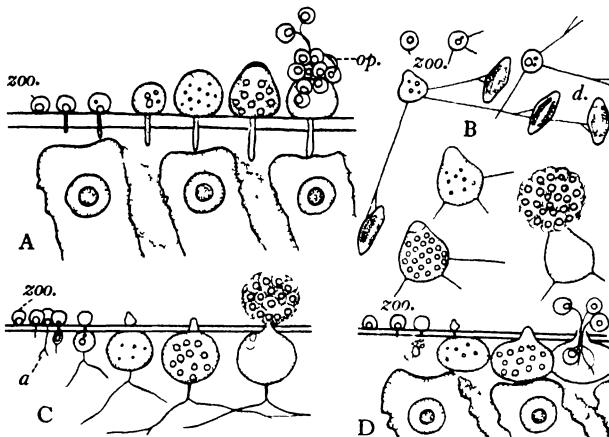
Fig. 1 Diagrams illustrating the types of development in chytridiaceous fungi

A *Chytridium*-type Successive stages in the development of the encysted zoospore (*zoo*) into a sporangium The last figure on the right illustrates the discharge of the zoospores from the sporangium after the dehiscence of an operculum (*op*) Fungus parasitising *Spirogyra*

B *Rhizidium*-type Successive stages in the development of the encysted zoospore (*zoo*) into a sporangium and the discharge of the zoospores of the latter These spores later break apart from one another and swim away individually The fungus is parasitising diatoms (*d*)

C. *Entophlyctis*-type of development Successive stages in the development of the encysted zoospore (*zoo*) into a sporangium, and the discharge of the latter The zoospores later break apart from one another and swim away (C, *a*, see p 166)

D. *Olpidium*-type of development. Successive stages in the development of the sporangium and discharge of the zoospores

type (Fig. 1 A) the zoospore upon coming to rest on the surface of the host cell, loses its cilium, encysts, and produces a germ tube which penetrates the wall of the host and forms within a branched or unbranched tenuous or often somewhat inflated rhizoid, the function of which is to absorb food materials from the host. Once established, growth is not only evident in the rhizoidal system but in the body of the zoospore which enlarges and ultimately becomes transformed into a single sporangium. The zoospores are cleaved out by a simultaneous process and at maturity are discharged through a pore formed (in *Chytridium* and certain other genera) upon the dehiscence of a cap or operculum (Fig. 1 A, *op.*). In type 2, the zoospore comes to rest in the water or matrix, often not in contact with the host cell.

After encystment (Fig. 1 B), one or more processes emanate from the body of the spore, the tips of which come into contact with the host (Fig. 1 B, *d*, diatoms) and usually only barely penetrate it. The nourishment thus obtained is transported to the spore body which enlarges and ultimately becomes transformed into a sporangium. In the *Entophlyctis*-type (Fig. 1 C), the zoospore, after coming to rest on the host cell, forms a tube which penetrates the wall through which the content of the spore passes to the inside. There, the naked mass enlarges, becomes surrounded by a wall, and is the fundament of the sporangium from which rhizoidal processes arise (see, however, p. 166). During maturity of the sporangium a relatively broad tube is formed which penetrates the wall of the host and through which the zoospores are eventually discharged. In the fourth, *Olpidium*-type (Fig. 1 D), development is similar to the preceding save that the sporangial fundament itself takes over the entire process of food gathering, no rhizoidal system being formed.

The first of the new types of development (which also represent new genera), *Rhizosiphon* (Scherffel, 1926*a*), approaches the *Entophlyctis*-type. The two opposing processes put out from the sporangial fundament are, however, never rhizoidal in nature as in *Entophlyctis*, but broad, tube-like, unbranched, and parallel with the long axis of the algal filament (*Filarszkya*). Further, the spindle-shaped sporangial fundament (prosporangium) does not become the sporangium but gives rise to an extramatrical somewhat pyriform body within which the zoospores are delimited (sporangium). Thick-walled, apparently asexually formed resting spores are also produced within the host cell. These, upon germination, produce an extramatrical sporangium. Essentially the same type of development has been described by Valkanov (1929) for a fungus termed by him *Hyphochytrium hydrodictii* n.sp., but which is probably a species of *Rhizosiphon*.

In *Physorhizophidium* Scherffel (1926*a*), a parasite of diatoms, a *Chytridium*-type of development is exhibited. The encysted zoospore produces a knob-like intramatrical portion which may occasionally be provided with rhizoids. The body of the spore becomes the sporangium, and between the base of this and the surface of the host cell another knob-like structure is formed which is regarded as an appressorium and which has apparently developed subsequently to the penetration of the host cell. While this sequence seems borne out by the absence of such "appressoria" in young stages, the writer cannot understand the function of an anchoring or attaching organ formed *after* the host cell has been penetrated.

Micromycopsis (Scherffel, 1926*a*), a parasite in the desmid *Hyalotheca*, has an *Olpidium*-type of development. The intramatrical thallus becomes invested with a somewhat thickened wall, the outer surface of which possesses (in *Micromycopsis cristata*) a helicoid series of spines. The wall of the alga is penetrated by a stout tube through which the content of the intramatrical portion (prosorus) flows. The latter rounds up at the orifice of the tube and forms a thick-walled body (sorus) which in *M. cristata* is also spiny. The sorus splits into several sporangia which in turn release their zoospores. The genus bears a close resemblance to *Synchytrium* and more especially to *Micromyces*.

Another genus erected by Scherffel (1926*b*), *Olpidiomorpha*, is *Olpidium*-like in

its features but possesses zoospores, the single posteriorly-directed cilium of which is attached to the forward part of the spore body. In other chytrids the cilium is usually observed to be attached to the rear of the spore. Further investigations may reveal other methods of attachment which may, as Scherffel points out, invalidate the genus.

Endochytrium Sparrow (1933*a*) probably possesses an *Entophlyctis*-type of development although early stages were not observed. The mature thallus resembles that of a large species of *Entophlyctis* save that there is a profuse development and ramification of the stout rhizoidal system on which more than one sporangium may be formed. In the latter respect it also resembles members of the Cladochytriaceae, but in contrast to the latter no turbinate cells or other enlargements of the rhizoidal system are formed. The zoospores are discharged after the dehiscence of an oper-culum. Another fungus of an *Olpidium*-type, which also discharges its zoospores after the dehiscence of an operculum, has been found by Sparrow (*loc. cit.*) in eggs of Rotifers and has tentatively been assigned to *Endochytrium*.

The same investigator (1931*d*, 1933*a*) has encountered on leaves of *Elodea* a fungus termed *Megachytrium* which possesses a remarkable degree of thallus develop-ment of a type closely approximating that found in the higher orders of the Phy-comycetes. The zoospore upon coming to rest on the surface of the leaf produces a broad undulating hypha which, as it elongates, expands and branches profusely. The ultimate branches of the mycelium show no evidences of tapering distally to delicate points as in rhizoids, but maintain their hyphal-like nature. They may fuse laterally with one another in a remarkable fashion. The mycelium may become intra-as well as extramatrical. On the broader branches large, fusiform-truncate, or irregular swellings, separated from the concomitant hyphae by cross-walls, may occur. These become either sporangia or thick-walled resting spores. The sporangia may also occur terminally in which case they are apophysate; opening of the sporangium takes place in an operculate fashion, and after discharge, new sporangia may form within the empty one ("proliferate"). The resting spores upon ger-mination form an extramatrical, operculate sporangium.

Physocladia, also established by Sparrow (1931*c*, 1932*b*), resembles a species of *Nowakowskiella* but possesses an inoperculate sporangium, in this respect being similar to *Cladochytrium*. However, it differs from both of these genera in the formation of a definite flask-shaped vesicle into which the zoospores pass at discharge and within which they undergo a prolonged period of violent swarming.

In *Scherffeliomyces* Sparrow (1933*d*, 1934*b*), a variation of the *Chytridium*-type is encountered. The zoospore comes to rest free in the water, produces a single germ tube which comes into contact with the host cell (resting cell of *Euglena*), and forms an inflated structure which apparently functions as an appressorium. Penetration of the host cell follows and a poorly developed, slightly branched, more often peg-like absorbing organ is formed. The appressorial structure then enlarges and at maturity becomes a large spherical sporangium to which is attached the germ tube and the now empty cyst of the infecting spore. Zoospore discharge is inoperculate. Thick-walled resting spores similar to the sporangia in position, and bearing the

appendiculate structure, are also formed. No evidence of sexuality in their formation was observed.

Rhizidiopsis (Sparrow, 1933*d*) also exhibits a variation of the *Chytridium*-type of development. The zoospore upon coming to rest on the host cell (the diatom *Melosira*) germinates, penetrates the alga, and forms within it a branched rhizoidal system. The body of the spore itself does not enlarge, however, but produces an apical or subapical finger-like process which expands and within which, in addition to the original body of the spore, zoospores are formed. These are liberated in an inoperculate fashion from the sporangium. Extramatrical, sessile, dark brown resting spores with crustose walls have also been found. These upon germination form a sporangium. In its developmental features the fungus is closely allied to *Podochytrium*, but, in contrast to the latter genus, it forms no sterile basal portion on the sporangium.

Sporophlyctidium (Sparrow, 1933*d*), a parasite of *Protoderma*, possesses a *Rhizidium*-type of development. The zoospore lying free in the water forms a relatively broad, isodiametric germ tube, the tip of which makes contact with the host cell. The body of the spore enlarges and becomes the sporangium. The zoospores, which are non-ciliate, are liberated through a single lateral pore. The genus seems allied to *Sporophlyctis* but does not form the elaborate, richly branched rhizoidal system of that fungus.

Septolpidium (Sparrow, 1933*d*), a parasite of diatoms, is *Olpidium*-like in its developmental features. However, the tubular thallus at maturity becomes septate and each of the segments functions as a sporangium.

In *Endoblastulidium* (Codreanu, 1931), a fungus parasitic in the coelomic cavity of certain species of *Ephemera* larvae, development is probably also *Olpidium*-like. The plasmodial, uninucleate thallus may become very extensive and is ultimately transformed into a walled, elliptical structure. It is then ejected through the anal opening by the muscular efforts of the host and outside in the water becomes a sporangium. The zoospores are liberated by a splitting of the wall rather than, as in *Olpidium*, by the formation of a discharge tube. In this connection it is interesting to note that de Plessis (1933), in an account of a species of *Olpidiopsis*, found in the rhizoids of a liverwort, states that in this fungus discharge of the spores through a tube or their liberation by a splitting of the sporangial wall was dependent upon the relative degree of moisture present. The latter method of discharge occurred generally in the water, the former in soil. It is possible the same thing holds for *Endoblastulidium*, which may be a species of *Olpidium*. References to a number of peculiar organisms of doubtful affinities, all inhabitants of insect larvae, may be found in Codreanu's paper and in that of Debaisieux (1919).

From these researches, which have brought to light a large number of fungi which represent not new species but new genera, it seems obvious that we are as yet little acquainted with the forms present in nature. This fact, it is hoped, will not only serve as a much needed check to pointless phylogenetic effusions, but may possibly assist in diverting this energy to the more pressing and certainly more fruitful need for further "explorations."

(3) Sexuality.

Relatively little is known of the sexual reproduction of the chytrids and the method whereby the resting stage—represented by a thick-walled structure with oleaginous contents and generally similar in shape and position to the sporangium—is formed has long been a matter of conjecture in certain genera. The investigations of the period preceding that taken up in this article not only established the fact that these fungi possessed a sexual phase but that this was of an extremely varied nature. In *Olpidium*, copulation of motile, isogamous gametes occurs (Fig. 2 A). The zygote ultimately encysts on the host, penetrates it, and forms the resting spore. Kusano (1929) has also ascertained that in one species (*O. trifolii*), the two fusing gametes arise from different gametangia. In *Olpidiopsis*, there is copulation of two or more multinucleate different-sized thalli, the protoplasm of the smaller one passing into the larger which then becomes the resting spore (Fig. 2 B, C). In *Polyphagus*, after the establishment of the thalli, one (generally, though not always, the smaller) puts out a tube which makes contact with the other. Into the more distal portion of this tube the contents of both of the thalli are accumulated and there is eventually formed from it a resting spore (Fig. 2 H, I). In *Zygorhizidium*, a *Chytridium*-like genus, a small thallus (the "male") puts out a tube which makes contact with another, larger thallus ("female"). Through this tube the contents of the male passes into the receptive thallus and the latter ultimately becomes the resting spore (Fig. 2 P, Q). In *Sporophlyctis*, two thalli of unequal size come into contact, a pore is formed between the two, and the plasma of the *larger* passes into the *smaller* which becomes the resting spore. In *Siphonaria* and, it might be added, in the closely related genus *Rhizoclosmatium* (unpublished), a very unique type of copulation occurs. The writer can confirm the observations of Petersen (1903) made on this sexual process over three decades ago. The two thalli make contact through their rhizoidal systems and the content of the smaller plant is transported to the larger which becomes the resting spore (Fig. 2 L, M). In *Zygochytrium*, a conjugation of hyphae occurs which results in the formation of a thick-walled spore precisely as in the Zygomycetes (Fig. 2 N, O). While the validity of Sorokin's (1874) observations have been subject to much criticism, mainly on the basis of the bizarre combination of a Zygomycete and a chytrid, no convincing evidence has as yet been put forward to refute them.

In recent years, largely through the work of Scherffel (1925b), attention has again been directed to a study of the sexuality of the chytrids. This investigator has confirmed the type of sexual reproduction found in *Zygorhizidium* and has also called attention to the formation of dwarf sporangia which he regards as vegetatively living male gametes. These might conceivably be male gametangia. In *Rhizophidium goniosporum* Scherf., he has discovered an undoubted case of sexuality in the formation of the resting spores of this large genus, which the present writer can confirm from observations on British material (unpublished). The male gamete (rarely two) attaches itself directly to the receptive thallus, communication is established between the two, and the contents of the attached, encysted male passes
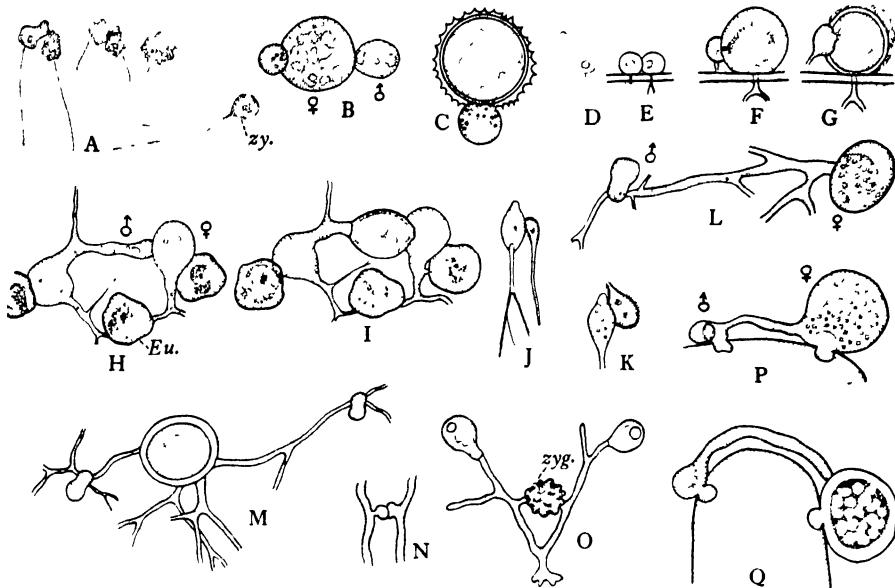
Fig. 2. Illustrating the types of sexual reproduction in certain chytridiaceous fungi.

A. Stages in the copulation of the isogamous planogametes of *Olpidium trifolii*. *Zy.*, motile zygote (after Kusano, 1929).

B. Large receptive thallus of *Olpidiopsis saprolegniae* with two "male" thalli.

C. Mature resting spore of same fungus with its companion ("male") cell (both after Barrett, 1912).

D. Swimming spores of *Rhizophidium* sp.

E. The same come to rest on surface of *Spirogyra* cell. The spores have encysted and produced the beginnings of a rhizoidal system within the alga.

F. Large receptive thallus with attached "male" cell; the latter has formed a fertilisation tube and has discharged its contents into the larger body.

G. Mature resting spore and attached male cell (all after Sparrow, 1933c).

H. Early stage in the conjugation of two thalli of *Polyphagus euglenae*, parasitic on *Euglena* sp. (*Eu.*).

I. Later stage of same, showing mature resting spore which has been formed in the distal portion of the conjugation tube from the contents of the two thalli (both original).

J. Conjugation of the thalli of *Sporophlyctis rostrata*, parasitic on *Draparnaldia*.

K. Same, showing binucleate zygote which in this case is the smaller of the two plants (both after Serbinow, 1907).

L. Conjugation through the rhizoidal system of two plants of *Siphonaria*. The contents of the smaller body could easily be observed passing to the larger one.

M. Mature resting spore of the same, showing in this instance two "male" cells which have discharged their contents into the larger plant (both original).

N. Early stage in the conjugation of the lateral branches of *Zygochytrium*.

O. Mature zygospore of same (*zyg.*), and habit of whole plant bearing two empty sporangia (both after Sorokin, 1874).

P. Conjugation of a small "male" plant with a large "female" thallus, found in *Zygorhizidium*. Parasitic on *Cylindrocystis*.

Q. Mature, thick-walled resting spore of same with male thallus and conjugation tube still attached (both after Lowenthal).

into the other in much the same manner as *Olpidiopsis* (Fig. 2 C). There is no loss of identity of the two copulating bodies, and after the receptive thallus has become transformed into a thick-walled resting spore, the empty male cyst remains adherent to it. This direct attachment of individuals, in contrast to the formation of a tube as in *Zygorhizidium*, seems to Scherffel to indicate a stronger sexual attraction of the spores for one another than was present in the last-named genus. That the two gametes are at first morphologically similar seems probable from observations on the aforementioned British material. From both Scherffel's and the writer's observations it is also evident that the receptive thallus may sometimes undergo considerable enlargement before contact with a male gamete. It would be interesting to study the development of the two in cases where contact with the opposite sex does not occur. In other species of the large genus *Rhizophidium*, Scherffel has observed indications of sexuality similar to that described above.

Couch (1932*b*) has briefly indicated that in a multiporous species of *Rhizophidium* a type of sexual reproduction similar to that of *R. goniosporum* exists. Sparrow (1933 *c*) has also observed a like case in what may be the same species. The zoospores which give rise to the copulating thalli are identical in shape and size. After a period of swarming they come to rest on the surface of the host cell (*Spirogyra*) in groups of 4–10 or more. The two adjacent spores each produce the usual rhizoidal system within the host. One spore now enlarges rapidly and extends its rhizoidal system, whereas the other apparently ceases to grow (Fig. 2 D-F). Ultimately, there can be observed within the larger thallus at the point of contact of the two plants a short refractive tube, seemingly formed by the smaller, male structure (Fig. 2 F). The entire content of the latter, with the exception of a small globule, is discharged through this tube into the larger plant which continues to increase in size and becomes transformed into a thick-walled resting spore (Fig. 2 G) to which the empty cyst of the male plant—generally torn from its rhizoidal system—remains adherent. In one instance, tubes of considerable length were noted on both large and small thalli, and while their function was not determined it might well be conjectured that under certain conditions the fungus may have the ability to form copulation tubes similar to those of *Zygorhizidium*.

In *Rhizophidium*, therefore, it is clear that we have a very definite sexual process involved in the formation of the resting spore, although many phases of this process need further amplification. Further, the type of sexuality found is a step removed from the isogamous planogametic copulation found in *Olpidium* (Fig. 2 A) and *Synchytrium*, in that at least one of the gametes is generally non-motile and has established a well-developed thallus before contact with the other takes place, and in most cases both have come to rest and germinated. In one instance a well-developed fertilisation tube is formed which functions and probably originates in the same manner as that found in the higher families of the Phycomycetes (Pythiaceae, Peronosporaceae). The formation of this structure would seem to remove this type of reproduction further from the *Olpidium*-type than superficial resemblances would indicate. Finally, the two gametes maintain their individuality after copulation, the one being represented by the mature resting spore, the other by the adherent cyst.

Resting spores without companion cells have been known for many years in the genus *Rhizophidium*, and their significance in relation to those just described awaits a satisfactory explanation. It seems scarcely probable that such obvious bodies as companion cells have been overlooked by all previous investigators. Indeed, resting spores of both types have been described in the same paper (Scherffel, 1925*b*; Couch, 1932*b*). It is possible that in certain species they are formed after a process of planogametic fusion similar to that of *Olpidium*, or, as Scherffel has suggested, parthenogenetically. From the specialised structure exhibited by most of the resting spores figured in the literature they can scarcely be regarded as "encysted sporangia."

Attention might also be directed to two other instances in which a sexual process in the formation of the resting spore may occur. In a fungus termed *Phlyctidium eudorinae*, Gimesi (1924) has figured what he interprets as a copulation of motile gametes. The ciliated zygote settles down on the host cell and becomes a thick-walled resting spore. While possibly suggestive of the type of sexuality found in this genus, which on the basis of its thallus and sporangial stage is very doubtfully distinct from *Rhizophidium*, the figures and evidence given are not convincing and need further elaboration. Couch (1931*b*) has encountered in *Micromyces*, a genus allied to *Synchytrium* but parasitic on fresh-water algae, swarm spores seemingly in the act of copulating with one another. As the material which formed the basis of these particular observations was killed and fixed, further evidence from living plants will be necessary to refute the possibility that they are not incompletely cleaved zoospores. However, from a comparison with *Synchytrium* in which such a copulation has been definitely proved, it is more than likely that a similar condition exists in *Micromyces*. While not within the scope of this paper, the highly interesting observations of Kusano (1928, 1929) on certain chytridiaceous parasites of flowering plants should be perused.

From the foregoing it may be seen that recent investigations have confirmed the findings of certain older observations on the process of sexual reproduction among the chytrids, and have established that a definite sexual process is involved in the formation of the resting spores of certain species of the large genus *Rhizophidium*. Evidences pointing to the existence of sexuality in the genera *Micromyces* and possibly in *Phlyctidium* have also been found. However, in over two-thirds of the genera of the Chytridiales evidences of sexuality are still lacking, and the resting spore, which in such ubiquitous forms as *Diplophlyctis*, *Chytridium*, and *Cladochytrium* is often found in abundance, is apparently an asexually formed structure.

(4) INVESTIGATIONS ON WELL-ESTABLISHED FORMS.

In addition to the discovery of new methods of development and hitherto unknown phases in the life history of members of the Chytridiales, several papers have appeared which have to do with the morphology and development of better known and well-established species. Some of these reiterate and confirm, often in a rather extensive manner, the findings of older investigators, while others contain new information concerning significant points overlooked or misinterpreted by previous

workers, points which in some instances have a very direct bearing on the relationships and affinities of the particular organism.

Weston's (1918) excellent work on the morphology of the saprolegniaceous zoospore laid a foundation for accurate and precise investigations of the morphology of this structure among other fungi, and has served to emphasise Butler's (1928) contention of its significance in determining affinities. While there are records in the older literature which would seem to indicate that the swarmer (and particularly its ciliation) is of a variable character in a given genus, this has been subsequently explained in all cases where it has been subjected to scrutiny either as an abnormal phenomenon or as a case of gametic copulation. Indeed, we may be reasonably safe in saying that in the ciliation of the spore, not only are all species of a genus alike but that all true members of an order possess spores with the same type of ciliation. Among the fungi commonly assigned to the Chytridiales, there are many which resemble one another to a marked degree in their thallus structure, but, because of the difference in the nature and ciliation of their zoospore, are evidently unrelated organisms.

Recent work has strikingly illustrated how a reinvestigation of the precise morphology of the zoospore can radically change our conception of the relationship of an organism. The genus *Ectrogella* has long been known in the literature, and while it has apparently been infrequently observed it has, from Zopf's account and excellent illustrations, been fairly well known to most mycologists. As described by him and included in even as recent a book as Fitzpatrick's (1930) on the Phycomycetes, it is evidently an *Olpidium*-like form which inhabits diatoms and which possesses a linear series of discharge tubes from which emerge posteriorly uniciliated, typically chytridiaceous zoospores.

Scherffel (1925a) has encountered a fungus which in all respects, save in the ciliation of the zoospores, exactly resembles Zopf's species. However, the zoospores were found by Scherffel to be definitely biciliate and diplanetic. Hence, he rightly regards *Ectrogella* as a saprolegniaceous fungus and not a chytrid. In further support of his contention he points out that the walls of the thallus turn violet with chloriodide of zinc, a reaction not encountered among the uniciliate members of the Chytridiales. Several similar instances might be cited. Sparrow (1934c) has reexamined a peculiar marine fungus, *Eurychasma*, parasitic on various brown algae, said by various investigators to possess a posteriorly uniciliate zoospore, and has ascertained from stained preparations that two cilia are present. This, together with the fact that the membrane of the thallus turns blue-violet with chloriodide of zinc, indicates, as in *Ectrogella*, that we are not dealing with a chytrid. Similarly, the same author (1934c) has also examined several marine species of *Pleotrachelus*, a genus supposedly allied to *Olpidium*, and has found that they, too, possess biciliate spores. These forms he has placed in the new genus *Petersenia* and considers them possibly related to *Olpidiopsis*. Puymaly (1927) has found that *Sphaertia*, a parasite of various Protozoa and generally regarded as being related to *Olpidium*, also possesses biciliate zoospores. These forms have been placed in *Pseudsphaerita* by Dangeard (1933). Cook and Nicholson (1933) have re-examined the zoospore of

Woronina polycystis and find that it possesses two apical, and not, as heretofore supposed, two lateral cilia. Further work on the zoospores of these fungi will undoubtedly reveal other discrepancies with descriptions and illustrations found in the older literature.

In a series of excellent observations on the development of *Catenaria anguillulae*, a parasite of liver fluke eggs, J. Bayley Butler and Buckley (1927) and E. J. Butler (1928) have given us a clear picture of the sequence of events both in the egg and in artificial cultures (J. B. Butler and Humphries, 1932). The thallus is established within the egg in a manner similar to that of a species of *Entophlyctis* (Fig. 1 C). At a point on the sporangial fundament nearly opposite the tip of the penetration tube a broad hyphal-like filament is produced. Subsequent portions of the thallus which develop from the primary swelling (sporangial fundament) are first laid down as broad, sometimes slightly inflated filamentous structures which subsequently become markedly swollen. Further observations (including those of E. J. Butler) indicate that the narrow, isodiametric isthmus which is often found connecting successive inflated elements of the thallus becomes separated by cross-walls from the swellings at a relatively late period. These swellings, as has been intimated, ultimately become the sporangia, each of which when mature possesses a single, generally unbranched discharge tube. Rhizoids may arise from either the sporangia or the isthmuses. The zoospores, which are completely formed within the sporangium, exhibit a short period of active swarming before discharge; upon the dissolution of the tip of the discharge tube they emerge rapidly, remaining a few moments in a compact spherical mass at the orifice of the discharge tube before assuming individual motion and darting away.

In artificial cultures (see p. 155) Butler and Humphries have found that the fungus within the infected egg emerges from the latter and produces a very extensive extramatrical growth. A single thallus may under these conditions form as many as twenty hyphal strands, each of which in one instance produced on the average three to four sporangia. In another case, over seven hundred sporangia were observed on a single outgrowth. From the variability in the shape of the sporangia formed under these conditions, it was concluded that this character was not influenced by environmental conditions but subject to some inner unknown control. In comparing the profuse development of the fungus obtained in artificial culture with the limited character of that formed in the eggs, Butler and Humphries feel that strong support is given to the idea advanced by certain older investigators that the simple structure exhibited by these fungi is a result of the degenerative influence of parasitism. However, since the fungus was said to grow equally well inside *boiled* fluke eggs, where presumably the same type of thallus developed as in live ones, the simplicity of the thallus appears to the writer as likely to be controlled by the limitation of space and available food imposed upon it, as by the cumulative effects of a parasitic mode of life.

Germination of zoospores and the formation of thalli and sporangia from these were also obtained in culture media by Butler and Humphries. Here the body of the zoospore itself became the initial sporangium and produced one or more hyphal

strands on which rhizoids and sporangia were formed. To the writer, the interesting feature of these observations was the transformation of the body of the spore itself into a sporangium. One would expect, as Karling (1930) found in another endophytic chytrid, *Diplophlyctis*, that some sort of filament corresponding to the penetration tube would first be developed, and that at the tip of the latter the sporangium would eventually be formed. Thus, in *Catenaria* when grown in nutrient solutions, the development is no longer of the *Entophlyctis*-type, but more closely resembles *Rhizidium* (Fig. 1 B). Indeed, were certain of the smaller thalli figured by Butler and Humphries found occurring in nature, they would doubtless be referred to *Rhizidium* or the allied genus *Rhizophlyctis*. Resting spores were obtained in only two instances by these investigators. They have, however, been found in abundance by Buckley and Clapham (1929) in eggs of *Dibothriocephalus*. In formation of the resting spores the contents of the sporangium contract and become surrounded by a stout wall on which, in the region of the discharge tube of the sporangium, there is formed a short pip. Upon germination the tip of the latter structure cracks open and a tube is formed which passes through the old discharge tube to the outside of the egg, and through which the zoospores, formed from the protoplasm of the resting spore, pass. While granting that such a body as described by them can rightly be called a "resting spore" and that as such is capable of withstanding a certain degree of desiccation, from the method of its formation, by reason of the fact that in the possession of a discharge tube it was already delimited as a sporangium and because of its simple structure, it cannot be compared with similarly named bodies found in such genera as *Rhizophidium*, *Chytridium*, etc., but should possibly be regarded as an encysted sporangium.

Karling (1928*a*, *b*) has re-examined carefully the development of several endophytic chytrids, all of which have an *Entophlyctis*-type of development. In *Entophlyctis heliomorpha* and *Diplophlyctis intestina*, both inhabitants of various Characeae, he has described essentially the same type of development as was heretofore known. However, in two subsequent papers (1930, 1931*a*) he indicates that, contrary to his previous observations, after penetration of the host wall, the content of the infecting zoospore does not at once form the spherical sporangial fundament from which rhizoids later arise, but that the rudiments of the rhizoidal system are first laid down (Fig. 1 C, *a*) and that the sporangium (and in *Diplophlyctis*, the subsporangial apophysis) is a secondary development. From Karling's previous observations and those of older writers it is possible that there may be some variation in the sequence of events in the establishment of sporangia and rhizoids.

Sparrow (1931*c*) has given an account of the initial stages in the method of infection and the establishment and development of the thallus of a species of *Cladochytrium* (*C. Nowakowskii*) found parasitic on *Spirogyra* and several other green algae. The thallus is formed within the alga in the same manner as in *Entophlyctis*. Karling (1931*b*) has encountered a closely related species (*Cladochytrium replicatum*) inhabiting the dead leaves and stems of various aquatic flowering plants and gives an extensive account of the mature thallus. In this genus, the well-developed rhizoidal system is characterised by the formation of septate, occasionally

non-septate, enlargements termed variously "Sammelzellen," "turbinate cells," "turbinate organs," etc., which Karling aptly points out form, with the sporangia, centres for reduplication or "replication" of the thallus. The somewhat dubious but highly useful term "rhizomycelium" has been applied by him (1931*b*, 1932) to this type of rhizoidal system. Sparrow (1931*c*, 1933*c*) has encountered resting spores in both *C. Nowakowskii* and *C. replicatum*. These were borne on the rhizomycelium in the same manner as the sporangia, i.e. as terminal or intercalary structures, and were relatively thin-walled bodies containing a large oil globule in their content. Those of *C. Nowakowskii* were smooth-walled, while in *C. replicatum* the wall was spiny. Karling (1934), having seemingly overlooked the previous description of resting spores in his species, has recently reported the discovery of similar spiny-walled spores in *C. replicatum*. Incidentally, in discussing the formation of resting spores in *Cladochytrium*, Karling asserts that we have at last in these relatively thin-walled spiny resting bodies and in their method of germination—transformation into a sporangium—as compared with the thick-walled, smooth ones of *Physoderma*, a clear-cut differentiation between the two genera. While not appreciated by the authors of various text-books on mycology and pathology, there have been since the 'seventies of the last century very evident differences between these two genera and no one who has worked with both forms considers it a difficult matter to distinguish them. In *Cladochytrium* the sporangia are formed on an intramatrical rhizoidal system which has been established within the host cell or substratum in an *Entophlyctis*-like fashion. On this intramatrical system, it may now be added, are also borne the thin-walled resting spores. In *Physoderma*, the intramatrical system has never been observed to produce any type of reproductive organ save the thick-walled, often elliptical and coloured resting spores. These upon germination produce sporangia, and zoospores which in turn come to rest on the surface of the host cell and form epibiotic sporangia which possess a somewhat bushy, rudimentary rhizoidal system. Such a phase has not thus far been encountered in a species of *Cladochytrium*. Sparrow (1934*a*) has established the fact that these epibiotic sporangia are also formed in two species of *Physoderma*, *P. zea-madyis* and *P. meny-anthis*, in which they were not heretofore known to exist, and has suggested that they will be found in all species of the genus. No evidences of sexuality in the formation of the resting spores of either *Cladochytrium* or *Physoderma* have been convincingly demonstrated. Ojerholm (1934) has suggested that certain biciliate zoospores formed upon the germination of the resting spores of *Physoderma zea-madyis* might be the result of a gametic fusion. Such spores have often been observed by the writer in this and other species of the genus (unpublished) and have been regarded by him as due to incomplete cleavage in the sporangium caused by unfavourable environmental conditions. If such a copulation occurs, as has been suggested by Ojerholm, it would seem more likely that the spores produced by the epibiotic "sporangia," which have been shown to be markedly smaller than those from germinating resting spores, would function in this manner (Sparrow, 1934*a*).

The fungus recently described by Cook (1934) as *Cladochytrium caespitis* Griffon and Maublanc, while resembling somewhat the description of that organism by its

authors, can hardly be considered a species of *Cladochytrium*. From Cook's figures and from an examination of material from the same source, it would seem to be a mixture of several organisms.

Couch (1931*b*) (see also Denis, 1928) has given an account of the development of *Micromyces*, a genus allied to *Synchytrium*, together with certain cytological details. The fungus, a parasite of *Spirogyra*, establishes itself in the host in the manner of an *Olpidium* (Fig. 1 D). Within, the amoeboid fungous mass moves toward the nucleus of the host, becomes closely appressed to it, and increases in size. The mature thallus is uninucleate. During growth, protoplasmic strands are developed which radiate from the main mass and attach themselves to all parts of the host cell, particularly to the pyrenoids. These attenuations ultimately become the long hyaline spines so characteristic of the prosorus. The latter after a short period of rest discharges its contents into a thin-walled, extramatrical vesicle (sorus). Since the protoplasm in this vesicle is multinucleate, the large single nucleus of the prosorus must have undergone division, although whether this occurred before migration of the protoplasm into the sorus or afterwards is not known with certainty. From comparison with *Synchytrium*, the latter condition is suggested. The contents of the sorus are then cleaved into 8–24 pyramidal sections with rounded bases and truncated apices. The nuclei of these sporangia undergo division, forming a large number of minute nuclei around each of which a zoospore is delimited. Emergence of living zoospores was not seen, although from the figures given they apparently escape through a pore formed in the truncated apex of the sporangium. Attention has already been directed (p. 163) to the evidences of sexuality in this genus. The resting spores, which may possibly originate from infection by larger spores (zygotes?) found in certain instances, are smaller than the sporangia and are surrounded by a thick, spiny, pale yellow-brown wall. Partial germination of one spore was observed; this process seemed to be similar to that encountered in the prosorus.

Mention might be made of Scherffel's (1926*b*) conclusive evidence affirming Pascher's (1925) contention that the supposed chytrid *Harpochytrium* is not a fungus but an apochromatic Heterokont. New species have been added by Scherffel (1926*b*) and Steinecke (1929).

Fragosa's (1925) genus *De Tonisia*, from the meagre description given, does not appear to be a fungus. Entz (1930) has described as *Oovarus copepodarum* n.gen. et n.sp. a parasite of copepods, a fungus said to have uniciliate zoospores. From the shape of these and from their method of formation, *i.e.* in a vesicle as in *Pythium*, they strongly resemble those of a species of that genus or of *Lagenidium*. Sassuchin (1934) summarises a number of papers on the parasites of various protozoans, the chytridiacean members of which are usually referred to the genus *Sphaerita*. Other papers on these rather aberrant forms are referred to in the papers of Mattes (1924) and Jahn (1933).

Briefer accounts of a large number of different chytridiaceous fungi from various parts of the world may be found in the following papers: Martin (1927), Jaczewski (1922), Köhler (1924), Scherffel (1925*a, b*, 1926*a, b*, 1931), Skvortzow (1925, 1927), Krafka and Miller (1926), Graff (1928), Matthews (1928), Buckley and Clapham

(1929), Lund (1930), Valkanov (1931*a*), de Wildeman (1931), Cejp (1932*c*, 1933), Couch (1932*b*), Dangeard (1932), Sparrow (1932*b*, 1933*a*, *c*, *d*, 1934*b*, *c*), Cook (1932), Tokunaga (1933), Rozsypal, J. (1934).

From a general survey of the papers on members of the Chytridiales it is obvious that most of them have been concerned with accounts of their occurrence, the discovery of new genera and species, a clearing up of moot points in the morphology and life history of a few forms, the beginnings of attempts to cultivate them on artificial substrata, and above all with a survey of what forms are actually present in fresh and salt waters. When compared with researches in the higher plants and even among other groups of the fungi, these investigations seem of a rather elementary nature, and so they must be until more concerted and continuous work gives us a fairly complete picture of what forms exist and methods by means of which we may maintain them in pure or even gross cultures.

III. BLASTOCLADIALES.

This small order comprises but two genera, *Blastocladia* and *Allomyces*. The former genus, of which there are eight species, is an exceedingly common inhabitant of submerged twigs and rosaceous fruits on which it forms crisp white pustules of closely crowded plants, often of only one species. A monographic account of the order has been published by Kanouse (1927), who has suggested the inclusion of a new genus, *Mindeniella*. Common characteristics of members of the order are the possession of a basal cell, anchored to the substratum by a root-like system of holdfasts, sessile sporangia in which are produced posteriorly uniciliate zoospores, and resting spores with peculiar punctate walls. On the distal portion of the basal cell in *Blastocladia* the sporangia and resting spores are borne in a sessile fashion. In *Allomyces,* this basal cell is not so pronounced, and from its apex the filamentous, branched mycelial portion of the thallus arises on which are borne the reproductive organs. In *Mindeniella*, the thallus resembles that of *Blastocladia*; the resting spores and sporangia, however, are borne on short pedicels and are delimited from the rest of the plant body by cellulin plugs. Further, the sporangia are spiny or smooth and the so-called resting spores are non-punctate and spiny. While Miss Kanouse considers her fungus a member of this order, the pedicellate nature of the reproductive organs, the blue colour reaction obtained after application of chloriodide of zinc (a reaction not found in *Blastocladia* and *Allomyces*), and particularly the ornamentation of the sporangia, seem to relate the plant more nearly to *Araiospora*, a Leptomitaceous fungus. While zoospore formation is stated to be "as in the genus *Saprolegnia*," no zoospores were evidently seen, and hence the most significant morphological feature in determining the affinities of *Mindeniella* is not known. If these, upon future investigation, are found to be uniciliate, the fungus is undoubtedly related to *Blastocladia*; however, if they are biciliate, there are strong reasons for considering it a species of *Araiospora*. Kanouse's paper and a preceding one (1925*a*) contain valuable notes on the collection and maintenance in gross culture of the inhabitants of submerged fruits. Neither *Blastocladia* nor *Mindeniella* has been successfully cultivated on artificial media.

The genus *Allomyces* occurs in water or soil and may be obtained on a variety of substrata. There have been described three species, *A. arbuscula* E. J. Butler (1911), *A. moniliformis* Coker and Braxton (1926), and *A. javanicus* Kniep (1929, 1930). In contrast to *Blastocladia*, it can be easily cultivated on a number of media.

One of the most outstanding contributions to our knowledge of the aquatic Phycomycetes has been the discovery by Kniep of anisogamous, planogametic reproduction and so-called alternation of generation in *Allomyces javanicus*. In this species, after copulation of the small, uniciliate male gamete (conspicuous by the possession of a red-orange oil globule) with the large, colourless, uniciliate female gamete, the biciliate zygote eventually comes to rest and immediately germinates. The resulting plant bears on it two types of reproductive structures, sporangia, and brown, punctate-walled resting spores. Uniciliate zoospores from these sporangia produce, upon germination, plants which also bear sporangia and resting spores. Dried resting spores after a period of several months may germinate if placed in pea decoction at 29° C. In this process, which takes 1–2 days, the spore is converted into a sporangium and the swarmers liberated upon germination produce plants which form in basipetalous succession chains of ovate, truncate gametangia. The latter, while resembling one another in shape, differ with regard to size, position, nature of their contents, and type of swarmer produced. The male gametangia in this species are generally terminal, and alternate in the chains with the larger female structures. The latter at maturity form a number of large, colourless, posteriorly uniciliate gametes which are liberated into the water, whereas the male gametangia produce a large number of relatively minute posteriorly uniciliate gametes, further distinguishable, as has been said, by the presence of a coloured oil globule.

Thus, in this species of the genus there would seem to be a definite morphological alternation of generations similar to that encountered in certain algae, although it is yet to be proved that this alternation is cytological as well. Two distinct plants are formed, one of which, originating from the zoospore liberated from the germinating resting spore, forms male and female gametangia, the other being derived from the quickly germinating zygote, and similar in superficial aspect, except that it forms sporangia and punctate resting spores. In contrast to other Oomycetes, as Kniep has pointed out, the zygote does not form a resting spore at once, but there is interspersed a thalloid development on the mature structure of which these bodies are borne. Since germinating zygotes and zoospores from sporangia produce only plants bearing sporangia and resting spores, it is obvious that reduction division does not occur in the first-named process or in the production of zoospores. Hence the sporangial plant must be diploid and reduction division must occur at the germination of the resting spore. A cytological examination of ungerminated resting spores shows them to contain many large nuclei. Preceding zoospore formation at germination, nuclear division occurs and many small nuclei are formed, one of which is contained in each zoospore. One of these divisions, Kniep considered, must necessarily have been heterotypic, although actual mitotic figures were not observed, nor chromosome counts made. While the nuclei of the supposed haploid generation were calculated to be smaller in volume than those of

the supposed diploid generation, variation in nuclear size would not seem to be an all-sufficient criterion, and Kniep's interpretation of the cytological alternation may not be borne out by chromosome counts. Indeed, in a paper given at the 1933 meeting of the Mycological Society of America, W. R. Hatch has reported that in a form, termed by him *Allomyces arbuscula*, the time when meiosis occurs may vary and may sometimes take place during the motility of the zygote. This, which was ascertained by observations on mitotic figures and chromosome counts, may account for the fact that certain species of the genus fail to show or exhibit only rarely the pronounced morphological alternation of generations observed by Kniep.

The main features of the life cycle of *Allomyces* as indicated by Kniep have been confirmed by Hatch (1933). From the appearance of the "secondary sporangia," figured by Coker and Braxton (1926) as occurring in *A. moniliformis*, it is possible that gametangia are also produced in this species.

From Kniep's work on *Allomyces* it is natural to suppose that a similar type of sexuality exists in the closely related genus *Blastocladia*. There is as yet no evidence to support this supposition. Kanouse (1927) has suggested that another type of reproduction may take place. In two plants of *Blastocladia globosa*, several slender filaments were observed which, because of the nature of their walls, did not seem to be parts of the basal cell and which, because of their greater diameter, could not be considered setae, structures commonly occurring in certain species. Further, their tips were markedly clavate and separated from the rest of the filament by a cross-wall. These swollen structures were regarded as antheridia and the punctate resting spores as mature oospores. No indication as to how these antheridia functioned could be observed, although in one instance it seemed that they might be similar in this respect to the antheridia of the higher Oomycetes. While the extreme scantiness of the data given are by no means sufficient to warrant Kanouse's assertion that true antheridia are formed in *Blastocladia*, nevertheless, these filamentous structures may possibly be gametangia of a sort.

Cotner (1930*a*) has given cytological details of the zoospores of *Blastocladia* (*B. Pringsheimii*, *B. globosa*), and has also ascertained that they have a very definite, narrow optimum range of temperature for discharge (11-14° C.). He also has determined that posteriorly uniciliate, uninucleate zoospores are the ones typically formed, although under unfavourable conditions, especially high temperatures, compound ones having more than one cilium and nucleus may be produced. His interpretation of the nuclear structures, particularly the blepharoplast, has been disputed by A. C. Mathews (1932) and by Hatch (aforementioned verbal communication) who confirms Mathews.

IV. MONOBLEPHARIDALES.

The members of this order, containing two genera, *Monoblepharis* and *Gonapodya*, possess a well-developed filamentous mycelium and are generally found occurring as pustules of delicate threads on submerged and sunken undecorticated twigs in cool, clear water. Such twigs even if showing no evidences of the fungus at the time of collection, if placed in sterile water and left for 3-7 days at 8-15° C. will generally yield *Monoblepharis* (Sparrow, 1933*b*). Material of *Gonapodya* is most

easily obtained from submerged rosaceous fruits (Kanouse, 1925*a*; Sparrow, 1933*b*). None of these forms has ever been grown in artificial culture.

Three papers of some extent have recently appeared which have to do with these fungi. That of Laibach (1927) is mainly concerned with the cytological aspects of *Monoblepharis* and *Gonapodya*; Barnes and Melville (1932) present excellent detailed observations on fertilisation in *Monoblepharis* and record the occurrence for the first time of members of the order in Great Britain; Sparrow's monograph of the order, based on observations of material from various parts of the eastern United States and Great Britain, contains an account of the morphology, development, occurrence, and distribution of the group, as well as a taxonomic treatment. Apinis (1930) in Latvia and Scherffel (1931) also report finding species of *Monoblepharis*.

It will be recalled that the outstanding feature of *Monoblepharis* is its method of sexual reproduction. Several small uniciliate sperm are produced in each antheridium, while a single uninucleate, non-ciliate, yet slightly motile egg is formed in the oogonium. The egg when mature possesses a prominent receptive papilla which, according to Sparrow, is a naked protrusion of the ooplasm but which, according to Barnes and Melville, is, when formed, covered by the thin wall of the oogonium. The latter investigators, in eight oogonia of *M. macrandra* studied by them, failed to find evidences of such a papilla. This point, which is at variance with other observers of this species, is of great interest, for it seems to indicate that there are strains in nature which approach even closer than we supposed the more primitive condition of an unspecialised female gamete, such as is found in *Allomyces*. Fertilisation in the genus *Monoblepharis* is accomplished by a sperm coming to rest on the receptive papilla and being rapidly absorbed into the ooplasm. The fertilised egg, which has moved toward the apex of the oogonium, then retreats to a more proximal position and remains motionless for a few minutes. It then expands and slowly moves out of the oogonium. Outside, this zygote normally remains attached to the mouth of the oogonium and soon surrounds itself with a thin pellicle which ultimately becomes the thick, bullate wall of the mature resting spore (Sparrow). In two species the zygote exhibits no motility, maturing within the oogonium into a smooth-walled spore (Thaxter, 1895). An interesting observation on the behaviour of the egg has been noted by Barnes and Melville. In one instance, before fusion of egg and sperm had been completed, the egg had started to evacuate the oogonium. This exceptional degree of motility has suggested to them a strong likeness to *Allomyces*. Indeed, in this character and in the non-papillate nature of the egg this strain of *Monoblepharis macrandra* approaches a very primitive condition and would warrant further study.

Laibach (1927) has confirmed the findings of Lagerheim (1900), that the egg is uninucleate and that after fertilisation fusion of the two nuclei occurs at the time of wall formation. He has further established that, at the time of the germination of the oospore, the single large resting nucleus is replaced by a number of smaller nuclei. When reduction division takes place is not known, as no mitotic figures were observed (save in one doubtful instance), but it is supposed to have occurred at the first division of the resting nucleus. These smaller, presumably haploid, nuclei migrate into the elongating germ tube.

With respect to the non-sexual stage, both Sparrow and Laibach present clear-cut evidence that the zoospores of *Monoblepharis* are uniciliate and borne in narrowly clavate sporangia, rather than biciliate and formed in oogonial structures as asserted by Thaxter (1895, 1903). Sparrow has given in detail the characteristic appearance and structure of these spores and points out their resemblance to those of *Gonapodya*, *Blastocladia*, and *Allomyces*. Cytological features of sporangial formation are given by Laibach for both *Monoblepharis* and *Gonapodya*.

No sexual stage has been convincingly demonstrated in *Gonapodya*.

V. SAPROLEGNIALES AND LEPTOMITALES.

The best known and most ubiquitous groups of the aquatic Phycomycetes, the members of these orders have been the subject of intensive study in recent years, and a number of papers have appeared concerning their morphology, occurrence, and, to a lesser degree, their physiology and cytology.

The order Leptomitales has been erected by Kanouse (1927) to include certain saprolegniaceous genera (*Leptomitus*, *Apodachlya*, *Sapromyces*, *Araiospora*, and *Rhipidium*), the thallus of which is divided by porous cellulin plugs into pseudo-cells. While similar thalli are encountered in both the Blastocladiales and the Mono-blepharidales, the grouping as proposed by Kanouse is a convenient one.

(1) OCCURRENCE AND CULTIVATION.

The members of these orders are usually saprophytes on a variety of plant and animal substrata, but may be hemi-parasites or occasionally, as in certain algal-inhabiting species of *Aphanomyces* (Couch, 1926a), seemingly obligate parasites. Most members of the two orders can be cultivated on artificial media (see, however, Kanouse, 1927; Kevorkian, 1934).

In recent years it has become evident that members of the Saprolegniales are not only of common occurrence in water, but that they are perhaps equally common inhabitants of soils. An elaboration of the technique used by E. J. Butler (1907) in connection with the isolation of species of *Pythium* from the soil has revealed a large phycomycetous flora (Harvey, 1925, 1927a, b, 1928a, b, 1930; Coker and Braxton, 1926; Coker, 1927; Couch, 1927; Raper, 1928; Apinis, 1930). The method of collection used is exceedingly simple: Soil samples from various localities and depths are placed in small bottles or envelopes; later, in the laboratory about 10 c.c. of the material is placed in a Petri dish and barely covered with sterile water. A small bit of nutrient substance such as an insect, or better, a piece of split hemp seed, is placed in contact with the soil. Free zoospores or ramifying hyphae may thus make contact with the substrate, and after a few days growth should be evident. The fungus is then washed clean of adherent soil particles and placed in a new dish where it is allowed to develop. (See also Addendum, p. 186.)

Interesting observations on the parasitic habits of certain saprolegniaceous fungi have come to light, particularly with regard to the genus *Aphanomyces*. Species of the latter have been found parasitic on algae, other aquatic fungi (Coker, 1923;

Couch, 1926*a*; Sparrow, 1933*c*), and flowering plants (Drechsler, 1929), while others have been found to possess the somewhat unique character of capturing and parasitising certain microscopic animals, notably rotifers. These rotifer-capturing forms have been segregated from *Aphanomyces* into two genera (*Sommerstorffia* Arnaudow, 1923; *Hydatinophagus* Valkanov, 1931*b*, 1933), but aside from their peculiar type of parasitism, encountered in other groups (Sommerstorff, 1911; Gicklhorn, 1922; Sparrow, 1929; Drechsler, 1933*a*, *b*, *c*, 1934), there seems little justification for this. In *Sommerstorffia* (Arnaudow, 1923; Sparrow, 1929), the mycelium is limited in extent and consists of a few tapering branches, the tips of which are spike-like. These tips secrete a mucilagenous substance by means of which the passing rotifer becomes stuck. Once captured, the animal generally fails, in spite of its efforts, to escape, and its body is soon invaded by the fungous mycelium. Ultimately, there is produced from the somewhat swollen intra-matrical mycelium a slender evacuation tube through which the zoospores are discharged. Parthenogenetically formed oospores have also been observed by Arnaudow.

Couch (1926*a*) in a series of interesting experiments on the range of parasitism of a species of *Aphanomyces* (*A. exoparasiticus* Coker and Couch), found in nature on *Pythium*, encountered seven types of interaction between host and parasite. These were derived from observations made on the fungi growing in plates of maize agar, and were characterised in part as follows:

(1) Hosts (saprolegniaceous and pythiaceous fungi) inhibited and much para-sitised, parasite not inhibited.

(2) Hosts (*Mucor* spp.) considerably or entirely inhibited, the parasite more or less inhibited; host threads parasitised.

(3) Mutual inhibition at point of contact; parasitism only along line of inter-mingling threads of the parasite and of other species of *Aphanomyces*.

(4) Prospective host (*Mucor* parasite *Syncephalus*) at first parasitised in zone of contact, later overgrowing the parasite.

(5) Mutual inhibition upon contact, no parasitism; occurring when two cultures of the parasitic *Aphanomyces* are plated together and also when *Sordaria* sp., *Asper-gillus niger*, and *Penicillium* spp. were each plated with the parasite.

(6) Prospective host (*Schizophyllum*) not inhibited, the parasite inhibited and unhealthy in vicinity of prospective host; hyphae of two not intermingling.

(7) Type of reaction depending upon temperature. The parasite plated with *Allomyces* at room temperature (21° C.?) covered the plate after two weeks nearly to the exclusion of the latter; the *Allomyces* heavily parasitised. Using the same com-bination at 34° C., after eight days the *Allomyces* had overgrown the *Aphanomyces*; no parasitism was observed. The same two fungi grown for two days at 34° C. and then placed at room temperature, after three weeks the good growth of unparasitised *Allomyces* which had previously developed was overrun and attacked by the *Aphanomyces*.

It would have been interesting to study the effect of temperature in other in-stances. These reactions seem to be further evidence that here, as elsewhere, an

organism existing under unfavourable conditions is more susceptible to attacks by parasitic forms, and further, that the virulence of the parasite itself is modified by environmental factors.

(2) NEW GENERA OF THE SAPROLEGNIALES.

It is to be expected that intensive work among a relatively little-known group of organisms will inevitably bring to light new forms, and that a re-examination of older-known ones will result in accumulating information which may alter our established concepts. Students of the Saprolegniales have been materially assisted in recent years by Coker's (1923) monograph of the group. In this volume a taxonomic account of nearly all of the species then known of the genera *Aplanes*, *Protoachlya*, *Leptolegnia*, *Saprolegnia*, *Pythiopsis*, *Isoachlya*, *Achlya*, *Aphanomyces*, *Dictyuchus*, and *Thraustotheca* is given, as well as certain genera of the Leptomitales. These descriptions are accompanied in most instances by illustrations. In addition, a large amount of miscellaneous data on collection, details of the morphology, variations of the latter on different substrata, seasonal distribution, etc., are interpolated; new species are described, and descriptions of the older species are in most cases based on material collected by the author and his students in the southern part of the United States (North Carolina). It would have been of interest to know whether or not the specific descriptions, obtained from pure cultures, were derived from single-spore pure cultures. Also, the discussions and interpretations of the various established species might have been greatly enhanced if type specimens, wherever these were available, had been consulted. While in certain cases this seems to have been done, the well-preserved and extremely important collection of de Bary's slides in the British Museum (Natural History), containing a number of saprolegniaceous and pythiaceous Phycomycetes—many of which, from a comparison of the dates of collection and publication, must certainly be considered types or co-types—was evidently not examined.

No doubt due to the stimulation for further work which Coker's book aroused, forms have since been found which in their method of non-sexual reproduction (the most significant character now used for distinguishing the genera) appear intermediate between well-established genera or exhibit a type hitherto not known to occur. As an example of the latter, the genus *Geolegnia* Coker (in Harvey, 1925) might be considered. Here, the fundament of the sporangium becomes swollen at intervals, and after being cut off by a cross-wall from its contiguous hypha the protoplasm of these swellings gradually becomes segregated into spherical spores. The latter are non-ciliate, often multinucleate (Couch, 1927), and are liberated from the sporangium only after the disintegration of the sporangial wall. Free in the water they sprout and give rise to the vegetative thallus. Sex organs of a saprolegniaceous type are also formed. Another new genus, *Calyptralegnia* Coker (1927), has been established to include a species previously placed in *Thraustotheca* (Coker and Couch, 1923). Here, the non-ciliate spores are ejected from the sporangium after the dehiscence of an apical cap. A third genus, *Brevilegnia* Coker and Couch (in Coker, 1927), has also been established to accommodate certain *Thraustotheca-*

like plants which show aberrancies in their method of spore discharge. Other species have since been added (Couch, 1927; Harvey, 1927a). This genus, on the basis of its non-sexual stage, appears to resemble closely *Thraustotheca*, although in contrast to it the zoospores, liberated upon the disintegration (not bursting) of the sporangial wall, are often quite variable in size and shape, and the oogonium is typically monosporic. Occasional sporangia similar to those of *Achlya* are formed in monosporic cultures of certain species (Couch, 1927). Further, the spores after liberation in some species give rise to germ tubes, in others each produces a single zoospore (Coker, 1927; Couch, 1927). While the writer is not inclined to minimise the differences between these new genera and established ones, differences which have seemed of sufficient import to warrant experienced investigators considering them worthy of generic rank, he finds great difficulty from descriptions in the literature alone in segregating *Brevilegnia* from *Thraustotheca* on the basis of sporangial discharge. In certain species of these genera and other older ones occasional instances of sporangial discharge of an *Achlya-*, *Thraustotheca-*, or *Dictyuchus*-type have been observed in cultures produced from single spores (Couch, 1927; Coker, 1927; Coker and Couch, 1924; Couch, 1931a). While these instances may be considered by some to be due to environmental conditions, the possibility of the occurrence of hybridism should not be overlooked.

Aphanomycopsis Scherffel (1925a), a parasite of diatoms, is essentially an *Aphanomyces* living under restricted environmental circumstances. There is formed within the host a scanty mycelium, branched and slightly inflated. The evacuation tube of the sporangium in the region where it passes out of the host cell possesses markedly thickened walls, denoted by Scherffel as the "Spreizapparat." The latter evidently forces the valves of the diatom apart. Several apandrous saprolegniaceous oospores are formed in a distended portion of the intramatrical mycelium.

Plectospira Drechsler (1927) also possesses a type of zoospore discharge and evacuation tube similar to that of *Aphanomyces*. However, at the base of the discharge tube and continuous with it there is formed a complex of intercommunicating, swollen, lobulate elements separated from their concomitant hyphae by cross-walls. Another species has been added by Drechsler (1929).

(3) REPRODUCTIVE PHASES.

(a) *Non-sexual reproduction.*

Details of the non-sexual stage of saprolegniaceous and leptomitaceous fungi, particularly the formation and discharge of the zoospores, have been given in various papers (Schwartze, 1922; Couch, 1924; Lounsbury, 1927, 1930; Cejp, 1931; A. C. Mathews, 1932; Höhnk, 1933; Kevorkian, 1934). Cotner (1930b) has also investigated the development and discharge of the zoospores of various Oomycetes at optimum temperatures for their formation, which appear to be different for the various species, and has also given cytological details of the active stages of the spores. The latter observations have been reinvestigated in part by A. C. Mathews (1932) who takes issue with Cotner on a number of points, particularly with the inter-

pretation of the blepharoplast. Lounsbury (1930) has studied the formation of the sporangia of *Isoachlya* and has ascertained the stability, under varying environmental conditions, of the character which distinguishes the genus from *Achlya*, namely, the formation of secondary sporangia by proliferation as well as by lateral branching.

Couch's paper (1924) contains certain points of special interest. Among the older investigators considerable ingenuity was oftentimes exercised to account for the behaviour of the discharging and discharged zoospores that occurred in certain genera. In *Achlya* and *Aphanomyces*, for example, the spores after emergence form a group at the mouth of the sporangium and undergo there a quiescent, encysted stage. There is ultimately liberated from each of these cysts a laterally biciliate zoospore. This clumping together of the spores was considered by Rothert (1887) to be due to the presence of a jelly-like substance, and by Hartog (1888) to the mutual attraction or "adelphotaxy" of the spores to each other. In both of these genera Couch has found distinct and clear-cut evidence that the zoospores are held together by definite threads. In *Aphanomyces*, after contraction, these threads soon disappear and the spores are then held together by the mucilaginous surfaces of their cellulose walls. No evidences of ciliary action in the evacuation of the spores were noted. Höhnk's (1933) paper is so obscured by an inadequate command of the language that the observations and conclusions are practically unintelligible. It is seemingly concerned with a homologisation of the swarm stages of various saprolegniaceous fungi and *Pythium*, based mainly on observations of several instances of repeated emergence of the zoospores after periods of rest and encystment. There is also an account of the behaviour of the zoospores of *Achlya racemosa* germinating under starved conditions, in which case the spores may emerge from the encysted stage as non-ciliate, amoeboid bodies.

Cejp (1932*b*) and Sparrow (1932*b*) have given brief accounts of certain leptomitaceous fungi, and Linder (1926), Kanouse (1927), and Kevorkian (1934) have described new species of these. Shkorbatov (1927) describes a number of new varieties of saprolegniaceous fungi.

(b) Sexual reproduction.

Couch (1926*b*) has established heterothallism, or better, dioecism in the ubiquitous water mould *Dictyuchus monosporus*. In plants bearing sexual organs, the oogonial and antheridial plants were isolated and grown separately, in which case oospore formation did not occur. When the two strains were grown together, wherever the hyphae of the two intermingled, sex organs were produced and mature oospores were formed. Contact of the two strains was necessary and seemed to be the only type of stimulation concerned in the process. Environmental conditions had only a secondary and variable effect on the production of sex organs. Intercrosses between the four recognised species of the genus were successful, and the resulting variations in morphological characters, hitherto supposed to be of specific significance, were so marked as to invalidate all but the type species of the genus (*D. monosporus*). Male, female, and neutral strains were found to occur in nature, as

well as one which formed its oospores parthenogenetically. The last-named oospores were germinated and crossed: (1) with a male strain, in which case the latter was stimulated to the formation of (functional?) antheridia which applied themselves to the oogonia produced by the parthenogenetic strain; (2) with an oogonial strain, in which case oogonia were formed on both, and in addition on the parthenogenetic strain functional antheridia were also produced. Thus the latter strain was shown to be inherently monoecious. Germination of the oospores formed by dioecious strains disclosed an interesting fact, namely, that parts of the mycelium formed by the germ hypha were male, parts female, and parts mixed. This seemed to indicate that sexual segregation took place early in the process of germination. If the germ hypha gave rise to a sporangium, this segregation probably occurred in the cleaving-out of the zoospores, since some of the latter were male, some female, and some mixed.

Dioecism has also been reported in *Achlya bisexualis* (A. B. Couch in Coker, 1927) and in the leptomitaceous fungus *Sapromyces reinschii* (Jordan in Sparrow, 1932*b*).

Homothallism or monoecism has been definitely proved in *Leptolegnia* (Schlösser, 1929; Couch, 1932*a*) and in certain species of *Saprolegnia*, *Achlya*, and *Protoachlya* (Schlösser, 1929). Schlösser (1929) has also given some interesting details of regeneration and "reversal" in the isolated, diclinous sex organs of certain homothallic species. At all stages in their development before actual fertilisation, the antheridia and oogonia may, by changing the environmental conditions, be made to reverse the sequence of their protoplasmic changes and hence to become again vegetative in character. Further, if sex organs are placed under conditions favourable for sporangial formation, they may, if the process of fertilisation has not been initiated, be converted into sporangia and produce functional zoospores. It has also been found that in certain groups of *Saprolegnia* in which species are differentiated mainly on the number of oogonia supplied with antheridia, that this character can be modified considerably in a single form by changing the temperature at which growth takes place. Other points of interest are given in Schlösser's excellent paper. Kanouse (1932) has also emphasised the physiological approach to a study of the members of the Saprolegniales, and has given definite proof, in the case of *Saprolegnia parasitica*, that this species, hitherto supposed to be sexually sterile, may, under proper environmental conditions, produce sex organs in abundance.

From the foregoing it may be seen that recent studies have added much to our knowledge of the occurrence, peculiar parasitic habits, forms present in nature, and reproductive phases of the Saprolegniales and to a lesser degree the Leptomitales. Dioecism (heterothallism) has been shown to be present in *Dictyuchus* and certain species of *Achlya* and *Sapromyces*; monoecism, in *Leptolegnia* and certain species of *Saprolegnia*, *Achlya*, and *Protoachlya*. Work on the experimental morphology of certain saprolegniaceous forms has been productive of interesting results, some of taxonomic significance, and has opened up a little-known field in the study of these fungi.

VI. ANCYLISTALES.

Little has appeared concerning this small and obscure order, most members of which are parasitic in various fresh-water algae. Attention might, however, be called to the genus *Lagena* (Vanterpool and Ledingham (1930)), a parasite of the roots of cereal plants in Canada, which appears to be a member of this order.

Scherffel (1925*a*) has described several new species of *Lagenidium* found by him parasitic in various diatoms, and brief accounts of established species are given by him, as well as by Graff (1928), Martin (1927), Sparrow (1932*b*), and Thompson (1934). Serbinov (1925) gives a short account of the morphology and biology of *Lagenidium sacculoides*.

VII. PYTHIALES.

Since Matthews' (1931) very recent monograph of *Pythium* covers the more essential papers that have been published on this genus, there seems no need to recapitulate them here. However, several interesting features have been brought to light by recent researches of which mention might be made. It has become apparent that there are a number of species other than the well-known *P. debaryanum*-type, which are parasitic in the roots of crop plants (Drechsler, 1925, 1928*b*, 1930). Further, there are undoubtedly a large number which parasitise fresh-water algae (Sparrow, 1931*a*, *b*; Matthews, 1931; Tokunaga, 1932), and in certain instances these have been found capable of parasitising under laboratory conditions the fruits and roots of various crop plants (Sparrow, 1932*a*). The finding of a marine species of the genus (*P. marinum* Sparrow, 1934*c*) would seem to indicate that there may be other members of the order in the sea. Other species have been found in soil (Cejp, 1931*b*).

Little work has been done on the other aquatic genera of the Pythiales, *Pythiomorpha*, *Pythiogeton*, and *Zoophagus*. Kanouse (1925*b*) has given an account of *Pythiomorpha gonapodioides* and has encountered the sexual stage of the fungus, not hitherto observed. The oospores are spherical, thick-walled, smooth, and, in only 10 per cent. of the number observed, provided with antheridia. The latter were diclinous in origin, solitary, and wound around the oogonium. A peculiarity of the oospore is the apparent absence in its content of any large oil globule, a structure found in most, if not all, species of the order. New species of *Pythiomorpha* have been described by Ito and Nagai (1932), and brief references are given to the occurrence of *P. gonapodioides* by Apinis (1930), Cejp (1932*a*), and Sparrow (1933*c*). Buisman (1927) and Drechsler (1932) are inclined to regard *Pythiomorpha* as a saprophytic species of *Phytopthora* mainly on the basis of its method of zoospore discharge and irregularities of the mycelium. Drechsler's frequently reiterated inferences concerning *Pythiomorpha* might be greatly enhanced if they were accompanied by his personal observations on a species of the genus as understood by him. From the ubiquity of *P. gonapodioides* in nature this would seem to be easily accomplished.

Drechsler (1932), in a paper describing a new species of *Pythiogeton*, found in decaying leaf-sheaths of *Typha*, has given an extensive account of the genus.

In *Zoophagus* (Sommerstorff, 1911), the sporangia of which resemble the filamentous type produced by certain species of *Pythium*, the rotifer-capturing habit is again encountered. In this instance, as in *Sommerstorffia*, the animals are captured by means of a mucilaginous secretion produced at the tips of the hyphal branches. The fungus has been discussed in some detail by Gicklhorn (1922), Arnaudow (1925), and Sparrow (1929).

<div align="center">VIII. SUMMARY.</div>

From the foregoing account of certain phases of recent researches on the aquatic Phycomycetes, it may be seen that these have been mainly concerned with a determination of what forms are present in nature. There probably exists a large aquatic flora with only a few scattered members of which we are now familiar.

Among the Chytridiales, considered the rarest and most perplexing of these fungi, the beginnings of attempts to cultivate them on artificial media have been made. New types of development have been found, many new genera and new species, occurring in both fresh and marine waters, have been described, and a reexamination of established forms has clarified certain moot points in their morphology and life history. The diversity of the sexual processes previously known to occur among the various genera has been reaffirmed, and in the large genus *Rhizophidium* definite sexuality has been discovered in several species.

In the small order of the Blastocladiales, in the genus *Allomyces*, copulation of anisogamous planogametes has been found to occur. There is exhibited by one species (*A. javanicus*) a morphological alternation of generations. Two plants are formed, which while they resemble each other in superficial aspect produce different types of reproductive organs, one, male and female gametes, the other, zoospores and resting spores.

Several papers have appeared which give details of the morphology and particularly of the process of sexual reproduction of *Monoblepharis*. The zoospore of this genus has been definitely determined to be posteriorly uniciliate. Although the members of the Blastocladiales and the Monoblepharidales bear little superficial resemblance to each other, the similarity of their zoospores and particularly of their methods of sexual reproduction shows them to be closely related.

The Saprolegniales have been found to be of widespread occurrence in the soil. Forms have been found which parasitise other water moulds, capture rotifers, and cause diseases of crop plants. New genera and many new species have been described. Detailed observations on the process of non-sexual reproduction in certain genera have shown that they possess certain optimum temperatures for the discharge of their zoospores. The hitherto unsatisfactorily explained clumping of the emerged zoospores of *Achlya* and *Aphanomyces* has been found to be due to the presence of delicate interlocking protoplasmic strands on the spores. Dioecism (heterothallism) has been definitely proved to exist in the genus *Dictyuchus* and in *Achlya bisexualis*. It has also been observed in *Sapromyces reinschii*. Monoecism (homothallism) has been found among certain diclinous species of *Saprolegnia*, *Achlya*, and *Protoachlya*. It has been found that the nature of the developmental process taking place in

already delimited sex organs may be considerably altered by changing the environmental conditions. A new order, Leptomitales, has been established to accommodate forms, hitherto placed in the Saprolegniales, in which the thallus is divided by porous cellulin plugs into pseudo-cells.

Little of interest has been published concerning the small order Ancylistales, save that a new genus, *Lagena*, parasitic in the rootlets of certain cereals, has been encountered.

Since a very recent monograph of *Pythium* has brought together the extensive literature of that genus it has seemed superfluous to include it here. Recent work has established that there are a number of species other than those of the *Pythium debaryanum*-type, which are parasites of flowering plants, that there are many others which parasitise fresh-water algae, and that certain of the latter have been found to be capable, under laboratory conditions, of parasitising the fruits and roots of a few crop plants. A marine species of *Pythium* has been found. Among the other genera of the order, *Pythiomorpha*, *Pythiogeton*, and *Zoophagus* (a rotifer-capturing form), little work has been accomplished, save the discovery of the sexual stage of the first-named genus.

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ADDENDUM

(Nov. 28, 1934; see p. 173.)

Very recently Lund (1934[1]) has published an extensive paper on Danish Phycomycetes, which is mainly concerned with an account of the species found and notes on their occurrence, particularly with reference to the *p*H of the water. The sites examined by him were divided into five types, viz. (1) highly acid (*p*H *c.* 3·5–*c.* 4·5), (2) slightly acid (*p*H *c.* 5·3–*c.* 6·8), (3) neutrally acid (*p*H *c.* 5·2–*c.* 7·5), (4) neutrally alkaline (*p*H *c.* 6·5–*c.* 7·7), (5) constantly alkaline (*p*H *c.* 7·0–*c.* 8·4). From a determination of the species present in waters of these various types (119 sites), Lund concluded that, with respect to the single factor *p*H, a great many forms occur in all types of waters, while others prefer strongly acid or neutrally alkaline or constantly alkaline habitats. However, since it was observed that material also grew well in gross laboratory water cultures where the *p*H was often very different from that encountered in the field, he feels that this factor is probably not a decisive one. Aside from the obvious necessity of the presence of a proper substratum, the amount of decaying organic material and the oxygen content appear to be potent factors in the determination of what type of fungous flora a habitat will possess. Temperature also seems to exert an influence on some species (especially the formation of reproductive organs) whereas light and the permanence of free water on the site are of little importance.

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[1] "Studies on Danish freshwater Phycomycetes and notes on their occurrence particularly relative to the hydrogen ion concentration of the water." *K. danske vidensk. Selsk. Skr.* (Natur. Math.), **9** (6), 1.

THE INVESTIGATIONS ON GROWTH AND TROPISMS CARRIED ON IN THE BOTANICAL LABORATORY OF THE UNIVERSITY OF UTRECHT DURING THE LAST DECADE

By F. A. F. C. WENT.

ABOUT 20 years ago Boysen-Jensen (1910, 1913) of Copenhagen and a little later Paál (1914, 1919) of Buda-Pest first acquainted us with the existence of what have since been termed growth substances, these being special cases of what are now known as phytohormones. From their investigations it became evident that in the grass seedling the tip of the coleoptile secretes a substance which exerts an accelerating influence on the growth of the plant.

In order to make clear the meaning of this fact it is necessary to point out that during the germination of a grass, such as oats or maize, a hollow cylindrical organ, closed at the top, emerges from the seed. This is called the coleoptile and in its cavity the first leaf develops. This leaf afterwards breaks through and from that moment the growth of the coleoptile stops. We must realise that this growth is brought about by the elongation of cells which were produced by cell division before or shortly after the coleoptile emerged from the seed. It must be understood from the beginning that all the phenomena of growth described in this article belong to the domain of cell extension; cell division is deliberately kept out of discussion here.

The investigators mentioned already showed that when the coleoptile of an oat seedling is decapitated growth stops, but that growth can be resumed if the amputated tip, or another tip, is replaced on the stump, even when a little gelatin is applied between the stump and the tip. If instead of gelatin a thin slice of mica is put between the stump and the tip, growth is not resumed, evidently because the growth substance secreted by the tip cannot pass through mica, whereas it easily diffuses through gelatin.

A convenient way for the detection of even small quantities of growth substance is to place the tip laterally on the stump. In this case one side of the decapitated coleoptile receives a greater quantity of growth substance, and hence grows faster than the opposite side, a curvature ensuing which is more easily observed than growth itself.

Many investigators, however, obtained contradictory results which were only explained by the observation of Söding (1925) and of Dolk (1926) that after removal of the tip by decapitation the stump regenerates growth substance in its apical part

in about 3 hours, so that after a certain time a decapitated coleoptile resumes its growth. Hence only such experiments as are performed within 2½ hours after decapitation give any evidence for the occurrence of growth substance.

A little later Dolk (1930) was able to show that after a first decapitation a certain amount of growth substance remains in the stump, but that this is consumed, and after a second decapitation the stump is really free from growth substance. Basing his work on these experiments, van der Wey (1931) worked out an improved method for determining growth substance, again using the oat seedling as test object.

The original method for obtaining the growth substance was due to F. W. Went (1927). He placed the tips of oat seedlings on slices of agar-agar. In this case the growth substance diffuses into the agar. After 1 or 2 hours the tips were removed and the agar was cut into small square blocks, which could be placed on decapitated oat seedlings. Here they caused a resumption of growth, which could easily be detected, particularly if the block had been placed laterally on the stump. From what has just been said it will be evident that this method has since been slightly altered in so far as the oat seedlings are decapitated twice. On the other hand it must be repeated that only those curvatures which become visible within 2½ or 3 hours after the last decapitation give any information concerning the occurrence of growth substance.

More important than the method itself is the fact that it became possible to measure growth substance quantitatively, since the angle of curvature of a treated oat seedling was proportional to this quantity. F. W. Went (1927) proved this in different ways, for example, by doubling the number of tips on agar. The most convincing proof, however, was one in which he placed blocks of agar with growth substance upon other blocks of the same size but without this substance. After a certain time for diffusion each of the two blocks contained one-half of the original quantity in the first block. In the same way he was able to carry on the dilution once more, getting one-quarter of the original quantity. Making use of these blocks, and measuring the angles of curvature on decapitated oat seedlings, he obtained the values shown in Table I:

Table I.

Number of tips	Time on agar 60 min.	Time on agar 30 min.	Time on agar 60 min. once diluted	Time on agar 60 min. twice diluted
6	11·2 ± 0·5	6·1 ± 0·4	5·5 ± 0·4	2·8 ± 0·8
12	17·1 ± 0·8	—	11·2 ± 0·4	5·8 ± 0·4

The proportionality in this and other experiments is obvious except for the larger quantities of growth substance. Here a different factor limits the larger curvatures. In order to measure these larger quantities it is necessary first to dilute them, so that the angle of curvature is below the limit.

The method of F. W. Went (with the alteration mentioned above) has been largely used in the investigations by Kögl, Haagen Smit and Erxleben on the chemical nature of the growth substance, which they have to a great extent eluci-

dated (see Kögl, 1933 *a, b, c*, 1934; Kögl and Haagen Smit, 1933; Kögl, Haagen Smit and Erxleben, 1933 *a, b, c*, 1934 *a, b, c, d*; Kögl, Haagen Smit and Tönnis, 1933). They used decapitated oat seedlings as test objects in order to determine the strength of each solution of growth substance. For this purpose the seedlings are cultivated in water in such a manner that their original position is strictly vertical. They are grown in the dark or in red light (during the last few years orange lamps are used, which serve the same purpose, but being brighter, make the handling of the oat seedlings easier) in a room where the temperature is kept constant, generally at 23° C. The humidity is also kept constant and rather high, the moisture being between 90 and 95 per cent. Here the quantity of growth substance which causes a curvature of the top of a decapitated oat seedling of 10' is called 1 unit, 1 a.e. (*Avena-Einheit*).

Kögl and his collaborators succeeded at last in obtaining the growth substance in the pure crystalline state and they named it "auxin." They first tried to get this substance from the tips of maize seedlings, but it soon became evident that the quantities there are so small (in oat seedlings about $1/20,000-1/90,000\gamma$ per mg. of top substance) that they would never have succeeded in getting sufficient material if no other source of auxin had been discovered. It soon became clear, however, that this substance is widely distributed not only throughout the plant world, but also in the animal kingdom, and that a source for great quantities of growth substance is human urine. Man secretes per diem about 1-2 mg. of auxin, and it was possible to concentrate this substance from the ether extract of urine. By a series of further procedures they succeeded in getting the pure crystals. This was possible because they were able to measure the strength of their different solutions by using oat seedlings as test objects. As these procedures belong to the domain of chemistry I will not enter into details and only mention that they succeeded in determining the formula.

Auxin is an organic acid, stable at high temperatures, having the formula $C_{18}H_{32}O_5$, in which there is an end chain of the constitution

$$-CHOH-CH_2-CHOH-CHOH-COOH,$$

connected with a ring of five carbon atoms with one double bond and two chains of isoprenerests:

$$\begin{array}{ccc} CH_3 & CH_3 & CH_3 \\ | & \diagdown & | \\ CH_3-CH_2 \quad CH-CH \quad CH-CH-CH_2-CH_3 \\ | \quad | \\ CH \quad C-CHOH-CH_2-CHOH-CHOH-COOH \end{array}$$
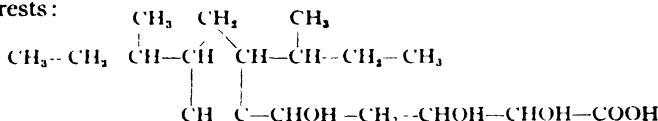
The question arose as to whether the substances extracted from urine, from yeast, from oat seedlings, etc., are identical. Until a short time ago it could only be said that no differences could be detected, principally with respect to the reaction of the plant cell. But it has now become evident that two other auxins exist. One of these has received from Kögl the name of *b*-auxin, in contrast to the *a*-auxin, already mentioned. Auxin *b* has the formula $C_{18}H_{30}O_4$; it probably is a keto-acid of the composition

$$R-CHOH-CH_2-CO-CH_2-COOH,$$

whereas auxin *a*, as was mentioned above, is

$$R-CHOH-CH_2-CHOH-CHOH-COOH,$$

a hydroxy-acid. The auxin lacton $C_{18}H_{32}O_4$, too, has an influence on the growth of oat seedlings, which influence, however, disappears after esterification of the acid. The supposition has been made that the double bond is the active part of the molecule. It is certain that after some time—after several weeks—the auxin, even at low temperatures and in the absence of free oxygen, loses its activity on the plant cell. This can perhaps be ascribed to an alteration in the double bond, to an intramolecular rearrangement.

But a third growth substance has now been discovered by Kögl and his school, which has a totally different composition. It is the already well-known substance β-indolyl-acetic acid, $C_{10}H_9O_2N$:

$$\begin{array}{c} \text{HC}\diagup^{\text{CH}}\diagdown\text{C}\!-\!-\!-\!\text{C}\!-\!\text{CH}_2\!-\!\text{COOH} \\ \mid\qquad\mid\mid\qquad\mid\mid \\ \text{HC}\diagdown_{\text{CH}}\diagup^{\text{C}}\diagdown_{\text{NH}}^{\text{CH}} \end{array}$$
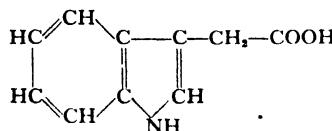
In order to distinguish this substance from auxin a and auxin b it has been called hetero-auxin.

As far as is known at present, these three substances exert the same influence on the elongation of plant cells, on phototropism and geotropism, so that it is not easy to differentiate them in small quantities. This differentiation has, however, been made by Kögl, who heated them either in acid or in alkaline solution. The three substances then behave differently. In the second instance it was possible to make an estimate of the molecular weight by following a method already used by F. W. Went (1927). Without going into details it can be said that the velocity of diffusion in agar-agar could be measured, and from this diffusion coefficient the molecular weight could be calculated.

The method used can, of course, only be trusted if the same quantity of auxin always gives rise to a curvature of the same amount. Though, as we shall see, this is not always the case, yet it may be said that in the same season of the year and at the same hour of the day, this quantity is practically constant. But obviously there are influences which alter the sensitivity of oat seedlings to auxin, so that in certain cases the same quantity of auxin gives a much larger reaction than in other cases. How is this to be explained, since conditions in the experimental rooms with respect to light, temperature and moisture are kept as constant as possible? As already mentioned, the sensitivity seems to differ in different seasons of the year, but sometimes it shows a certain daily periodicity, often with 2 maxima and 2 minima in 24 hours. Haagen Smit (see Kögl, 1933a, b) was able to destroy this periodicity by putting the seedlings in metal boxes, or rather in boxes made of a good conductor for electricity, so that electric fields were withdrawn and influences due to ionisation of the air done away with. It was irrelevant whether a box of zinc with a wall having a thickness of 0·8 mm. was used or one of lead of which the wall had a diameter of 5 cm. Accordingly there can be no question of the direct action of cosmic rays.

Under the auspices of Kögl investigations are being carried out by Haagen Smit,

with the help of J. J. Went. Preliminary results with auxin *a* suggested that the cause of the differences in sensitivity are to be found in very feeble electric currents. These workers have produced artificial differences of potential in the plant. When the top of an oat seedling was made positive with respect to the root substratum by connection with an external potential difference, the transport to the base could be accelerated to as much as 120,000,000 a.e. per γ. On the other hand, it was possible to retard it by a current in the opposite direction to about 10,000,000 a.e. per γ. In order to obtain the same deviation of the standard as those under natural conditions it was necessary to use currents which were of the order of 10^{-8} ampere.

The whole question seems very complex. In how far the variations of the standard tests are connected with different degrees of ionisation of the air remains to be seen. The connection certainly is not so direct as was first supposed. These investigations are being continued, as well as others in which the differences of electric potential in the oat seedlings themselves are measured.

It has already been mentioned that the presence of growth substance has been demonstrated in numerous cases in very different living organisms; in plants, in the first instance in the tips of grass seedlings (*e.g.* in maize in much larger quantities than in oats), in seedlings of dicotyledonous plants such as *Lupinus* spp., *Vicia faba*, *Raphanus sativus*, *Lepidium sativum*, etc. Growth substance has been detected in the leaves and stems of many plants; for example, a short time ago by Czaja (1934) in shoots of many trees, such as horse chestnut, pine and fir. The same may be said of the flower heads of the Compositae, as Uyldert (1927) has demonstrated, in the pollen of orchids (Laibach, 1933) and of many other plants (Thimann and F. W. Went, 1934), and in the cells of some algae, as *Valonia* (van der Wey, 1933).

Nielsen (1930) was the first to find growth substance in cultures of fungi, particularly in *Rhizopus suinus*. For this reason he called the substance "rhizopin," though he did not succeed in obtaining it in a pure state, so that it was not possible to distinguish it from growth substances derived from other sources. Afterwards yeast also proved to be a source of growth substance, as well as other fungi and several bacteria, such as *Bacillus coli*. It would carry me too far afield to enumerate all cases in which growth substance has been found in higher and in lower plants, but so far as we now know it rather seems as if growth substance is to be found throughout the vegetable kingdom, perhaps no single species being without it.

The question arises as to whether all these growth substances from different plants are identical. It is extremely difficult to give a definite answer to this question in the present state of our knowledge, so long as the growth substances have not been extracted, purified and analysed in each case. We may say, however, that their action on the plant cell is identical in all cases investigated. I have already mentioned that Kögl and his collaborators have been able to distinguish between auxin *a* and auxin *b*, the former being a hydroxy-acid, the latter a keto-acid, and hetero-auxin, which is identical with β-indolyl-acetic acid. I have also given a short indication of the way in which these three growth substances can be recognised. Though not many cases have been examined as yet, I can give the results of investigations (Kögl and Kostermans, 1934), according to which the growth substance

in the tips of grass seedlings (maize) is auxin *a*, whereas cultures of yeast, of *Aspergillus niger* and of *Rhizopus nigricans*, yield only hetero-auxin.

I have not yet spoken of the growth substance of roots because of the controversy on this subject. Cholodny (1923), Keeble, Nelson and Snow (1931) and others have obtained growth substance from root tips and other parts of roots, whereas Gorter (1932) could not find growth substance in root tips of *Zea mais* and *Pisum sativum*. Though the reason of these divergent results is as yet not quite clear, some light has been thrown on the question by Boysen-Jensen (1933). He succeeded in getting growth substance out of roots of *Vicia faba* when he put the tips on agar-agar with glucose, whereas no auxin diffused into pure agar. In repeating his experiments, Gorter obtained the same results. An explanation of this phenomenon has not yet been given.

On the other hand, it must be said that there is always some possibility of auxin being present in a form not easy to detect, namely as an ester. In this combination it is present in some seeds, from which the free acid can be extracted by proper treatment, either with dilute mineral acids, or better still by splitting up the esters with lipase.

In the animal kingdom auxin has been detected in many cases, though generally in rather small quantities. Tissues of dogs in particular were investigated by Kögl, Haagen Smit and Erxleben (1933*a*). Just as in men, dogs secrete a rather large quantity of auxin in the urine. The question of the source of this auxin arose. From experiments carried out by Haagen Smit (see Kögl, 1933*b*) in which he regulated his own diet, it was found that large quantities of auxin only occurred in the urine after a meal in which vegetable fats were present. If the fat was treated in such a way that the auxin was extracted before the meal, then this substance was not to be found in the urine. The conclusion may be that the auxin excreted with the urine does come out of the fats contained in the food. But these fats generally contain much auxin *b* and very little auxin *a*, whereas in the urine there is more auxin *a*. So it became evident that either somewhere during the course of digestion this auxin *b* is changed into auxin *a*, thus:

$$R.\text{CHOH}-\text{CH}_2-\text{CO}-\text{CH}_2-\text{COOH}$$
$$\rightarrow R.\text{CHOH}-\text{CH}_2-\text{CHOH}-\text{CHOH}-\text{COOH},$$
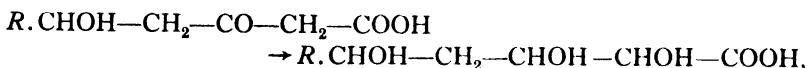
or that it is destroyed in the human body.

During the last few months Kögl has found that every urine also contains hetero-auxin independent of the food, but generally only in small quantities. It looks rather as if this β-indolyl-acetic acid is formed by the micro-organisms of the intestinal tract out of tryptophane or similar substances.

Perhaps it is worth while to mention that in malignant tumours no excess of auxin could be detected; reports to the contrary were shown to be erroneous by Kögl, Haagen Smit and Tönnis (1933), Kögl (1934). They probably were the result of a confusion with the " bios " of Wildiers, which is also secreted by yeast and which may perhaps be identical with Nielsen's rhizopin *B*. Once again it must be pointed out that the auxins have no influence on cell division, but act exclusively on the swelling of the cell.

The question now arises as to how the growth substance can exert an influence on the elongation of cells. Here, as in all the investigations yet to be mentioned, auxin *b* was occasionally used, but most of the experiments were made with auxin *a*. Up to the present no differences can be detected and the same must be said about the hetero-auxin, though this substance has seldom been used in its pure state.

It soon became evident that auxin exerts an influence on the cell wall. After a preliminary examination by de Haas (1929), Heyn in particular (1930, 1931*a*, *b*, 1932*a*, *b*, 1933*a*, *b*, 1934*a*, *b*, *c*), and also Heyn and van Overbeek (1931), made a thorough examination of this question, which, though not yet finished, led to certain conclusions, which in the beginning seemed to be almost identical with those arrived at by Söding (1931). The latter has afterwards, however, arrived at somewhat different views (1933*a*, *b*, 1934).

The conclusion of Heyn is, in brief, that auxin has an influence on the cell wall, the extensibility of which is increased. I shall leave out of discussion whether this influence is a direct one, or whether it is not much more probable that the growth substance alters the permeability of protoplasm and that in this way it makes possible the passage of enzymes or other substances which in their turn act on the extensibility of the cell wall.

Experiments were made both with coleoptiles of oat seedlings and with hypocotyls of *Lupinus albus*, with flower stalks of *Tulipa* and with internodes of *Vicia faba*. The extensibility was measured either directly by hanging small weights upon strips of tissue and by measuring the extension after a certain time and the length after the weight had been removed, or indirectly by bending the organs while they were in a horizontal position by putting a small weight on the free end and by measuring in the same way the curvature before and after removal of the weight. In other experiments the plants were forcibly bent through a known angle. The permanent curvature after removal of the bending force was then measured. All these investigations were carried out with and without growth substance, either in the growing stage or at low temperatures in order to stop the growth altogether.

We may say that the chief conclusion is that auxin has an influence on the extensibility, more especially on the plastic extensibility, of the cell wall. As long as a certain osmotic pressure exists in the interior of the cell, elongation must follow on this increase of extensibility, an elongation which is irreversible.

Recently Heyn has been continuing these investigations, partly in Paris and partly in Leeds in the Laboratory of Textile Physics and in Prof. Priestley's laboratory. He has been able to distinguish between the plastic elongation and the elastic extensibility, showing the former to be independent of the latter, but to be on the other hand largely dependent upon the action of the growth substance. The cell walls of the coleoptile of *Avena* consist of layers of different extensibility, the outer layers having a greater elastic extensibility than the inner ones. The fixation of the elastic extension can be explained by a relaxation of the outer layers due to the influence of strain. It is a process which is absolutely different from the irreversible

extension due to the influence of growth substance. X-ray investigations of the cell walls of the epidermis cells of oat seedlings are in progress; the preliminary results are very promising.

Heyn has now taken up the problem of the influence of growth substance on the growth of roots. The investigations of Cholodny (1923, 1926, 1928, 1929, 1931), of Keeble, Nelson and Snow (1931) and others led to the conclusion that growth substance retards the growth of roots. This has recently been confirmed by Laibach and Kornmann (1933a, b). Laibach made use of a new method of handling auxin. He succeeded in taking up the growth substance in an ointment, such as lanolin. He found that when he applied this ointment to the outside of intact plants he got results which could be explained by assuming that this auxin enters into the cells. If he applied lanolin only to one side of the oat coleoptile there resulted a curvature away from the auxin. On the other hand, if he made this same experiment with roots, then the curvature was positive, i.e. in the direction of the auxin, furnishing proof of the retardation of growth under the influence of growth substance.

The most direct proof of this has been given by Kögl and Haagen Smit in experiments which have just been published (1934b). When they cultivated oat seedlings with their roots in water to which a small quantity of auxin, either a or b, had been added, the growth of the roots was inhibited. They became thicker and were densely covered with root hairs. The phenomenon was visible when as little as 1γ auxin was given per litre of water, but it became much more conspicuous when 10γ was added. The same phenomenon could be observed with hetero-auxin. If roots are growing downwards in a moist room, it is curious to see that the moment they reach such a solution of auxin they do not enter it, but try to grow along the surface of the solution as if this contained some poisonous substance.

It remains to be seen how these different reactions of stems and roots to growth substance can be explained. Provisionally it gives the impression that in roots auxin causes an extension of the transverse cell walls, such as is known in the cases of contraction of roots investigated many years ago by de Vries (1880).

Very little is known about the production of growth substance. The plants which have hitherto been made use of in most of our experiments, namely oat seedlings, have a centre of production in the tip of the coleoptile and the same can be said for other grasses. This centre seems to be confined to the extreme half-millimetre of the tip. Perhaps cells with much cytoplasm in the tip may be regarded as some sort of glandular cells, secreting the growth substance. This was first suggested by Lange (1927) and later du Buy (1933) pointed out the probability of this explanation. After removal of the tip, the production of auxin is naturally stopped, but we have seen already that the apical cells of decapitated oat seedlings, which in normal plants never produce auxin, may regenerate this substance.

Such sharp localisation of the production of auxin as is found in grass seedlings is rarely found among other plants. In seedlings of *Raphanus* and *Lepidium* the centre of production is to be found in the cotyledons; according to van Overbeek (1933) the quantity of auxin rapidly diminishes in the dark, whereas in the light it is produced again, so that probably photosynthesis plays a part in its production here.

In the stem and leaves of *Vicia faba* van der Laan (1934) found great quantities of auxin everywhere. Thimann and Skoog (1934) have shown that it is produced in the young leaves during the sprouting of the buds and that here also light influences the production, just as in *Raphanus*. Etiolated seedlings of *Lupinus*, on the contrary, do not show a definite centre of production. It looks as if every cell were capable of producing auxin, perhaps from stored materials. It is certain that in some seeds which do not contain auxin in the free state, growth substance is present in an esterified form in which it is inactive as regards plant cells, but from which active growth substance may be obtained by the action of lipase. The experiments of Laibach (1933) show that pollinia of orchids contain very large quantities of auxin, the action of which had already been investigated many years ago by Fitting (1909). Fitting was also the first to use the term hormone for these substances.

It will be clear from what has been said that we are at present very ignorant about the mode of formation of auxin in the plant and a general investigation in this respect might give us many new and interesting results. It will then be necessary to take into consideration the existence of different auxins, since we know at least three. It has already been stated that up to the present there is no evidence for the production of auxin in the animal body. There is considerable probability that wherever it is detected in animal products its source in the last instance is a plant.

The transport of growth substance has been investigated by van der Wey (1932) in the coleoptiles of *Avena*. In order to do this he made use of the following device: Cylinders of different lengths were cut from a coleoptile and placed upon blocks of agar-agar without auxin. On the top of these cylinders blocks of agar with growth substance were applied. It was necessary to keep this whole arrangement at constant temperature, and also at high humidity because otherwise the agar-agar desiccated and extracted water from the coleoptiles. After a certain time the lower blocks were analysed so that the amount of auxin carried through the cylinders could be measured, or rather the quantity carried through minus the amount retained in the cylinders. By testing the quantity of auxin in the upper block before and after the experiment, the amount retained in the cylinder could also be determined. It must be said that considerable difficulties are incumbent to this procedure, and that it requires great dexterity on the part of the investigator. Leaving the method, however, let us look at the conclusions arrived at by van der Wey (1932).

The velocity of transport is so great that it cannot possibly be a simple physical diffusion. The living protoplasm must play a rôle here, particularly since the influence of temperature is generally in agreement with what would be expected if this were the case. I will not insist upon this, however, since the strongest argument for the action of living protoplasm lies in the fact that auxin can easily be transported from the top to the base, but not in the opposite direction, the transport being thus strictly polar.

This polarisation subsists even at 0° C., though one gets the impression that here the transport is a diffusion process. On the other hand, van der Wey (1934) did show that narcotising cylinders of the coleoptile with ether abolishes this polarised state; but if the concentration of the ether has not been too strong this is a reversible process.

But as soon as we try to form an hypothesis to explain the observed facts, we have to grope our way in the dark. F. W. Went (1927) and Brauner (1922) have suggested that protoplasmic streaming may have something to do with this transport. Van der Wey (1932) holds a different opinion: he believes in a transport either in the limiting layer between protoplasm and cell wall, or through the cell wall itself. This last hypothesis he founded upon the ideas of Wiesner (1892), who, many years ago, as is well known, maintained that the cell wall of the living cell consists not only of the cell-wall substance (cellulose, etc.), but also of minute particles of living protoplasm.

However this may be, the controversy induced Bottelier (1933, 1934) to carry out investigations on the influence of external factors on the protoplasmic streaming in the epidermis cells of the coleoptile of *Avena sativa*. He found that with young seedlings (at the age of about 90 hours) the Q_{10} is 1·8, but only between 7 and 17° C. If, however, this value is determined between 17 and 35° C. the velocity of streaming appears to be constant. On the other hand, if the experiments are carried out with older seedlings (at an age of about 140–200 hours) the Q_{10} between 7 and 25° C. is 1·8; above this last temperature the value diminishes. Now, in comparing for these coleoptiles the velocity of streaming at 16·5 and at 24° C. Bottelier obtained values of 10 and 21·3, whereas van der Wey got values for the velocity of transport of auxin at these same temperatures of 10 and 20. The agreement between these figures is such that there is every reason to suppose that some connection between the two phenomena may exist.

It may be of some interest to mention the results arrived at by Bottelier in his experiments on the influence of light on protoplasmic streaming. A short illumination calls forth after 3–4 min. a retardation, which lasts for about 4 min. The amount of retardation is dependent on the quantity of light. Large quantities of light (800 ergs per sq. cm.) may even give an acceleration. If the action of light of different wave-lengths is examined, the maximum effect is found with about 4500Å. (blue light), diminishing to zero both in the ultra-violet and at the other end of the spectrum, so that red and orange light are inactive.

It is also worth while mentioning that the intensity of the streaming shows large fluctuations during the year as well as during the day. It looks as if there may be some connection with the varying sensibility to auxin mentioned above. Here, however, one must certainly be extremely cautious in drawing conclusions.

I know very well that even if we consider protoplasmic streaming as the cause of the transport of the growth substance, it is impossible to explain in this way the polarity of the transport. This polarity was not only observed by van der Wey (1932) for oat seedlings, but also by Dykman (1934) for seedlings of *Lupinus* and by Laibach and Kornmann (1933a) for *Avena* seedlings, with their method described above of auxin absorption by the intact plant. F. W. Went has tried to give an explanation for this and other phenomena of polarity by differences of electrical potential between the two ends of each cell, resulting in the bottom of the coleoptile being positive to the tip. This potential difference would favour the transport of negative ions from the top to the base. On the other hand, positive ions would

travel with greater facility in the opposite direction. It must be borne in mind that the three known auxins are acids.

For many years it has been a well-known fact that ethylene exerts an influence on the growth and curvatures of many seedlings. It was of interest to determine whether this influence has anything to do with auxin. The question was examined by van der Laan (1934). He worked with seedlings of *Avena sativa* and of *Vicia faba*, and he demonstrated that ethylene has no influence either on the transport or on the consumption of auxin, but that the production of growth substance is inhibited by this gas. In this way the action of ethylene on growth could be explained. Also the geotropic sensibility of *Vicia faba* is diminished, and hence an epinastic movement can manifest itself, leading to what has been called horizontal nutation. But space does not permit me to go further into details here. However, it is worth while saying that van der Laan compares the influence of ethylene on the formation of auxin with the influence on enzymatic processes, so that he thinks there is some reason to bring the production of growth substance under this same heading.

We will now turn from the simple phenomena of growth to the curvatures produced under the influence of light or gravity, respectively called phototropic and geotropic curvatures. We shall first confine ourselves to phototropism. We speak of phototropism when curvatures occur whose direction is determined by the direction of the incident rays of light. Let us take the best known object in this respect, the oat seedling. We then see that it generally bends towards light; it shows positive phototropism. This is the case with small and with very large quantities of light. In between there is a zone where the oat seedlings bend away from the light; they show negative phototropism.

It has long been known that these curvatures are brought about by an unequal growth of the two sides of the coleoptile. Blaauw (1914, 1915, 1918), following an old theory of de Candolle, has attempted to explain phototropism with the aid of the so-called photo-growth reactions of the two sides of the seedling.

A digression is necessary in order to make this point quite clear. Blaauw investigated the reactions of growing organs with respect to light incident from all sides, or at least from several opposite sides so that no curvatures could occur. He showed that in the sporangiophores of *Phycomyces* the influence of light consists in an acceleration of growth. Both the amount of acceleration and the moment at which it sets in are dependent on the quantity of light given. He expressed the quantity of light as the product of the intensity of the light by the duration of the illumination, in so-called metre-candle-seconds (m.c.s.).

In other cases, especially with seedlings of higher plants, the primary effect of the light is a retardation of growth which may be followed by an acceleration. The extent of this retardation and subsequent acceleration depends upon the quantity of light. Blaauw thought it obvious that when a plant received light only from one side, the illuminated side should give a different photo-growth reaction from the shaded side, because part of the light going through the plant would be absorbed and another part reflected. Thus a curvature should ensue and here then we would have the cause of phototropism.

It is well known that a long controversy arose concerning this theory of Blaauw and the connection between the photo-growth response and phototropism generally. The strongest argument which Blaauw gave in favour of his theory certainly was the fact that such roots as carry out a photo-growth reaction are also sensitive to one-sided light, in that they show a phototropic curvature, whereas roots which are not phototropic do not show any photo-growth reaction.

The most complete set of experiments concerning the photo-growth reaction was carried out by van Dillewyn (1927) with oat seedlings. He gave a very complete set of records of the light-growth response with different quantities of light, and he was able to show that the tip of an oat seedling behaves in a different way from the rest of the coleoptile, let us say the base. In assuming that only 1/30 of the light on the front side arrives at the shaded side in one-sided illumination, he was able to calculate what should be the result of the co-operation of two light-growth responses with these different quantities of light. The result would, of course, be a curvature, and van Dillewyn showed that the direction of this curvature is completely in accord with what Arisz (1915) found many years ago in his experiments concerning the influence of different quantities of light on the phototropic curvatures of oat seedlings.

Although the directions of the calculated curvatures were the same as those actually observed, the calculated amounts were smaller than those recorded. This was pointed out by F. W. Went (1927). Others had made similar remarks, but he went further and proposed another explanation of phototropism. Whereas in light coming from all sides the transport of growth substance in oat seedlings goes on equally on all sides in a longitudinal direction from the tip to the base, an illumination from one side brings about a lateral transport of auxin in such a way that the greater part is moved to the shaded side which consequently will grow faster, hence a positive curvature ensues.

By placing the tip of an oat seedling, which had been illuminated from one side, on two blocks of agar-agar which were separated by the blade of a safety razor, it was possible to collect the growth substance from the two sides of the seedling separately and to test these two quantities by means of decapitated oat seedlings. Table II gives the results. The illumination was carried out with 1000 m.c.s. and the quantity

Table II.

Numbers	Time on agar min.	Light side	Shaded side	Sum
360–363	70	38	57	95
364–367	60	26	51	77
368–374	90	6	62	68
379–381	80	32	60	92
383–388	120	33	57	90
Average	84	27	57	84

of auxin in the dark was made equal to 100. Of the 84 per cent. of auxin left, 65 per cent. is to be found on the shaded side and 35 per cent. on the front side. This difference is, of course, quite sufficient to explain the positive phototropic curvatures.

On the other hand, the same table shows a diminution, due to illumination, of

the total amount of growth substance given off by the tip. In this instance about 84 per cent. remained, whereas 16 per cent. disappeared. In other experiments, where the light arrived in a longitudinal direction, this figure was about 18 per cent. The last word has certainly not yet been said here, for du Buy (1933) could find no such diminution in his experiments, although van Overbeek, in experiments which have not yet been published, is more nearly in agreement with F. W. Went. It should be borne in mind that it is not easy to determine very slight differences in the amount of auxin present in the tips of seedlings. However this may be, there is no possible doubt about the uneven distribution of the growth substance under the influence of one-sided light.

These two explanations of phototropism stood side by side until van Overbeek (1933) succeeded in making a synthesis of the two, principally based on experiments with seedlings of *Raphanus sativus* (a few experiments were also carried on with seedlings of *Lepidium sativum*). At first he obtained the same result as had F. W. Went for oat seedlings, that is, one-sided light caused the growth substance to pass to the shaded side. When a cylinder of the hypocotyl was provided unilaterally at the top with a block of agar-agar containing growth substance and when the auxin transported through the cylinder was caught separately on two sides of the cylinder, then of course the greater quantity should be found on the side where the block with auxin was applied. This is only true when the experiments are carried out in the dark or in light arriving transversely from all sides or going uniformly through the cylinder in a longitudinal direction.

If we have to do, however, with transverse light which is one-sided, then it becomes possible to catch the greater amount of auxin on the shaded side, even though the block of agar-agar is applied on the light side. This is only so when we use white light, or better light containing blue and violet rays, whereas orange light acts as darkness.

Table III gives the result of some of van Overbeek's experiments with seedlings of *Raphanus sativus*. In the first column the quality of the light is indicated, in the

Table III.

Light	Energy	Light one-sided		In the dark	
		Light side	Shaded side	One side	Opposite side
Orange	976,000	7·0 ± 2	6·0 ± 0 8	5·5 ± 0·8	5·0 ± 0·9
Orange	976,000	7·2 ± 2	6·0 ± 1·4	—	—
Orange	976,000	4·6 ± 2	2·6 ± 1	9·1 ± 0·9	7·1 ± 1·8
Orange	976,000	7·4 ± 1·9	5·8 ± 0·8	—	—
White	976,000	6·8 ± 0·6	12·1 ± 1 5	—	—

second column the quantity of light which is always the same here, namely 976,000 ergs per sq. cm. The next columns give the quantities of auxin caught on the light side and on the shaded side, while the last two give these quantities for plants kept in the dark. The figures are quite clear. In white light the growth substance passes to the shaded side, so that the transport instead of going in a longitudinal direction goes obliquely to the opposite side. This, then, is the same phenomenon as that

discovered by F. W. Went with *Avena* seedlings. It is noteworthy that orange light
has no influence at all, only rays of short wave-length being active. The same may
be said about the influence of rays of different wave-length on phototropism, a
further reason for adhering to this explanation of phototropic curvatures.

Van Overbeek also discovered another effect of light, for which it is irrelevant
whether this light is one- or all-sided. This effect is a diminution of the sensitivity
towards growth substance of the cells of seedlings of *Raphanus*. The same quantity
of growth substance causes a greater elongation of the cells in the dark than in the
light, probably by an action of light on the cell walls. Here we have the light-growth
reaction of Blaauw and it became possible to combine the effect of both light
reactions. In one-sided light we then have to do with a deviation of the growth
substance to the shaded side, and, moreover, with a diminution of the sensitivity
to this growth substance, which diminution is larger on the front than on the back.
Hence a curvature must ensue, as the combined effect of these two actions. Van
Overbeek even attempted with success to deduce the amount of this curvature
quantitatively from the two effects, although this will not be gone into in detail here.

A somewhat similar explanation, though not attended with the same success, was
later given by du Buy (1933) for the phototropic curvatures of the *Avena* coleoptile.
He did succeed, however, in explaining the so-called first and second positive curva-
tures as well as the negative response. According to his researches this question is
even more complicated, although we will not here go into it further. Thimann and
Skoog (1934) found that for *Vicia faba* also, light in some respects inhibits the action
of growth substance. Recently van Overbeek took up the study of these phenomena
in oat seedlings. He has not yet published any of the results and his investigations
have not yet come to an end. It may already be said, however, that here too light
from all sides reduces the sensitivity of the cells to the growth substance. This is
particularly the case when the light is not very strong and the reaction is more
especially one of the base of the coleoptile. As already stated, his experiments are
now going on and it is better therefore to say no more about them at present. But it
is certain that every investigation carried on in this direction with such a common
laboratory plant as the oat seedling promises a great many interesting results.

Another tropistic curvature, explanation of which has become possible on the
basis of growth substance distribution, is geotropism. It is to Cholodny (1923,
1926, 1928, 1929, 1931) that we owe the fundamental theory of this phenomenon,
but it was Dolk (1930) who gave the conclusive experimental evidence. Recently
his conclusions have been confirmed by the investigations of Dykman (1934).
Dolk carried out his observations with oat seedlings, whereas Dykman worked with
seedlings of *Lupinus albus* and *L. luteus*.

Since Darwin's classical book on the movements of plants, it has been known
that oat seedlings from which the tip has been cut off fail to respond to gravity
when they are placed in a horizontal position. Rothert (1896) was able to confirm
these observations and he also found that after a certain time the sensitivity for
gravity returns. The explanation could only be given after the growth substance and
the place of its formation had been discovered, because through decapitation of an

oat seedling the centre of growth substance production is removed and only after the regeneration of new auxin does the possibility of a geotropic curvature return. Dolk (1926) showed that geotropic sensitivity returns at the same time as does the regeneration of growth substance. F. W. Went (1927) replaced the tip of a decapitated oat seedling by a block of agar-agar with auxin. He was able to demonstrate that seedlings treated in this way can give a geotropic curvature. A short time ago Kögl repeated this same experiment with the same result, using, however, hetero-auxin instead of auxin *a* or *b*.

Dolk (1930) made use of a similar device to test the sensitivity of the different zones of an oat seedling to the stimulus of gravity. He decapitated coleoptiles at different heights and replaced the tip by a block of agar-agar with auxin. Oat seedlings treated in this way were placed in a horizontal position and the time necessary for a subsequent curvature just visible to the naked eye determined (the so-called presentation time). The result is given in Table IV, from which it is obvious that the tip is about 25 times as sensitive as a zone at a distance of 10 mm. from the top. Such a diminution of sensitivity, though to a much greater extent, had formerly been detected for phototropism.

Table IV.

Length of tip removed mm.	Presentation time in min
0 (control)	4
1	5–10
2	15–20
3	20
4	20–25
5	35
6	35–40
7	60
8	70
9	80
10	100–110

Much more interesting, however, was the question of why in oat seedlings which are placed in a horizontal position the lower side grows faster than the upper side, so that a negative geotropic curvature must ensue. Dolk (1930) also gave here a solution which was in accordance with Cholodny's theory. The direction of the transport is altered. Instead of flowing in a longitudinal direction, the growth substance is deviated in such a way that the underneath part of the coleoptile receives more auxin than the upper side.

Dolk first determined the amount of growth substance in tips of oat seedlings which had been kept in a horizontal position for a certain time, taking care to gather the auxin from the upper side and from the lower side in separate blocks of agar-agar (separated also by a safety-razor blade). He found that the quantity of growth substance in the lower half is larger than that in the upper half. Consequently growth will be faster on the lower side and hence a negative geotropic curvature will result.

I give here a few figures (Table V) obtained by Dolk for maize seedlings, where the differences between the upper and the lower sides are even larger than with the coleoptiles of *Avena*. The tips were placed on agar for 60 min.

Table V.

Time in horizontal position sec.	Number of tips	Quantity of growth substance	
		Upper side	Lower side
2700	5	12·4	19·5
1800	4	9·6	14·0
1800	4	10·5	16·2
1800	4	13·4	16·7
1800	4	8·3	22·5
1800	4	6·3	11·2
1800	4	2·5	8·5
900	4	4·6	8·7
1800	4	2·6	6·6
900	4	2·5	5·3
1800	4	3·2	7·0
1800	6	12·7	14·6
1800	6	12·3	16·7

On the other hand, Dolk placed oat seedlings in a horizontal position for half an hour and let them rotate afterwards for 60 min. on the horizontal axis of a klinostat. The differences of the two sides had then disappeared. As a result the geotropic curvature, even if it had started, began to go back and was no longer visible.

Table VI shows the figures which Dolk obtained by using seven tips for each experiment and letting these stand on agar-agar for 90 min. The first column gives the growth substance in the upper side; the second one that in the lower side:

Table VI.

5·8	5·6
6·7	7·0
6·8	6·6
6·3	5·6
11·0	10·6
12·0	12·5

Dolk carried on another set of experiments in which he made use of small cylinders cut out of oat seedlings. On one side of these cylinders a block of agar with growth substance was applied, on the other side there were two separate blocks of agar without auxin. When these cylinders were kept in a vertical position the amount of auxin arriving in the two blocks by transport through both sides of the cylinder was equal. But when the cylinders were placed in a horizontal position for 2 hours the quantities shown in Table VII were found, the first column giving the upper, the second the lower side. Here too the flow of the growth substance was deviated towards the lower side of the coleoptile.

If we take into consideration that the sensitivity to one-sided light diminishes much more rapidly from the top to the base of an oat seedling than the sensitivity

to the stimulus of gravity, and that in the latter case the auxin is deviated in its longitudinal flow not only in the tip, but also in the more basal zones of the coleoptile, it becomes clear why the geotropic curvature not only becomes visible much sooner than the phototropic, but also why it is that the former curvature travels to the base with much greater velocity.

Table VII.

6·3	9·8
7·5	10·3
4·0	8·8
6·5	8·0
6·3	8·8
3·4	6·8
3·8	8·8

In the past year Dykman (1934), working with very different material, namely seedlings of *Lupinus albus* and *L. luteus*, fully confirmed the experiments of Dolk with oat and maize seedlings. In *Lupinus* also the current of auxin is deviated when the hypocotyl is placed in a horizontal position. Dykman even made a calculation of the curvature which should result from the difference in the quantities of auxin found on the two sides of the seedling. The calculated values were in agreement with those actually observed; in fact the coincidence was very striking. Van der Laan (1934) in his investigations on the action of ethylene on plants also made the observation that in stems of *Vicia faba* the one-sided distribution of growth substance in seedlings placed horizontally is perceptible after three-quarters of an hour, or just at the time when the geotropic curvature begins.

In an investigation on the geotropism of the nodes of grasses, Schmitz (1933) found that here too the production of growth substance is influenced by gravity. It remains to be seen whether this same phenomenon can be found in other cases; we must take into consideration that the nodes of grasses occupy a rather exceptional position. We have here to do with intercalary growth, so it is perhaps worth while to mention the experiments carried out by Uyldert (1931) on *Tradescantia*, where it became obvious that auxin is also connected with transverse geotropism, although she has not yet succeeded in giving a complete analysis of this phenomenon. With regard to the longitudinal component of the stimulus due to gravity we have an investigation by Pfältzer (1934), who was unable to find any relation between this component and the distribution of growth substance.

Regarding positive geotropism I have already mentioned the investigations of Cholodny (1923, 1926, 1928, 1929, 1931) which have given us the following hypothesis. Here growth substance retards growth. Now, supposing that here also a deviation of the transport of auxin to the lower side of a horizontally placed root takes place, the result must be that the upper side grows faster than the lower side, hence a deviation towards the earth, a positive geotropism, must result. Though several facts point to this explanation, many more observations are certainly needed.

Naturally the question as to the reason of this deviation of growth substance to the lower side of horizontally placed organs arises. Brauner (1927, 1928) has discovered the geoelectric effect. This consists of a difference of electric potential between the upper and the lower side of horizontally placed plants. He has been carrying on experiments in the same direction with some of his students, and it is not at all improbable that the distribution of the acid, auxin, is related to this effect. Theories have been set forth, but I would rather restrict myself here to facts and leave hypothetical considerations out of the discussion. For the future, however, this line of research certainly promises a great deal.

There is one other matter which I should like to mention here in a few words, namely autotropism. If an organ which has responded either to one-sided illumination, to gravity, or to any other stimulus, is placed on a klinostat with horizontal axis, the curvature disappears, the organ stretches itself. Of course this is only the case so long as the curvature is not yet quite fixed by growth, but it can be observed in any phototropic or geotropic curvature. With the coleoptile of *Avena*, for instance, this curvature begins at the top and moves gradually toward the base. At the same time autotropism begins to show itself at the top which straightens out. This straightening follows the curvature so that in the end the curvature is only located at the base of the coleoptile. Descriptions of this phenomenon have been given many times, for example by Rothert (1896) and by Arisz (1915).

Dolk (1930) asked himself if this might not be explained by a connection between autotropism and growth substance. Indeed, such a connection was discovered by comparing intact coleoptiles with those of which the tip had been cut off after stimulation by gravity. In the latter case autotropism did not start until growth substance had been regenerated, although Dolk could hasten the onset by the application of auxin in agar-agar.

For the manifestation of autotropism, then, the presence of growth substance is necessary, and Dolk attempted to give an explanation of the phenomenon. When a curvature takes place, the convex side of the organ grows faster than the concave side because it possesses a larger quantity of growth substance. At the same time it uses up not only auxin, but also the necessary foodstuffs for the formation of new cell walls, etc. After a certain time these substances will be found in greater quantities on the concave than on the convex side. If new growth substance now arrives in equal quantities on the two sides, the result must be that the concave side will now grow faster than the convex, and consequently that straightening of the organ will set in.

Another definition of autotropism might be that it is the disposition of the organ to adhere to its own form; so that we have thus solved a morphological question. I know very well that this is one of the simplest of the form problems, but this suggests that the study of growth substance may in the future help us to solve one of the most complex questions of the living world.

My retirement as director of the Botanical Laboratory of the University of Utrecht has offered an opportunity for me to give a comprehensive review of some

of the work of this laboratory in the domain of growth and movements of plants. Space does not permit of a more extensive treatment, but this has already been done by others, particularly by Nuernbergk and du Buy (1932) and du Buy and Nuernbergk (1932, 1934).

Even in this short review it has been impossible to restrict myself to the work carried out in the Utrecht Laboratory. It was necessary first of all to include also the work of the organic chemistry laboratory of Utrecht University under the direction of Prof. Kögl, which is in such close co-operation with the work of the botanical laboratory. So much is done now in this domain in other laboratories that I have had briefly to mention several of these investigations, but I have restricted myself as much as possible in this respect, and hence do not in the least aim at completeness.

SUMMARY.

1. Growth substance can be extracted out of tips of oat seedlings by placing them on slices of agar-agar.

2. The quantity of growth substance can be measured by placing blocks of agar-agar containing this substance on decapitated oat seedlings, which then resume growth. If this is done on one side a curvature ensues and the angle of deviation is proportional to the quantity of growth substance, provided that this quantity is not too large.

3. It is necessary to take into account the fact that in decapitated oat seedlings regeneration of growth substance takes place after $2\frac{1}{2}$ or 3 hours.

4. By this method it became possible to concentrate and purify the growth substance, which received the name auxin. Three different auxins were detected: auxin a, a hydroxy-acid $C_{18}H_{32}O_5$, auxin b, related to the first named, a keto-acid $C_{18}H_{30}O_4$, and hetero-auxin, which is identical with β-indolylacetic acid $C_{10}H_9O_2N$.

5. As far as is known up to now, these three auxins have the same effect on the elongation of plant cells.

6. Growth substance is widely distributed throughout the vegetable kingdom and is also to be found in animals and their excretions, *e.g.* human urine. But it looks as if in animals the source of the auxin is always the plant taken as food.

7. The fact that the sensitivity of oat seedlings is not always the same, even in the dark in a room of constant temperature and constant moisture, may perhaps have some connection with the degree of ionisation of the air.

8. Growth substance has no influence on cell division; it only exerts its influence on cell extension by increasing the plastic extensibility of the cell wall.

9. Auxin retards the growth of roots, probably by causing an extension of the transverse cell walls, thereby shortening the cell.

10. The production of growth substance is sometimes related to photosynthesis, but fungi and bacteria can also produce hetero-auxin.

11. The transport of growth substance is strictly polar, going from the tip to the base, never in the opposite direction; it is limited to the living protoplasm.

12. Narcotisation with ether abolishes the polarity; this process is reversible.

13. There exists a connection between the transport of growth substance and protoplasmic streaming.

14. The production of growth substance in oat seedlings and in stems of *Vicia faba* is inhibited by ethylene.

15. Phototropism can be explained by means of the distribution of growth substance and by the influence of light on the sensitivity of the cells towards this substance.

16. One-sided illumination causes an unequal distribution of the growth substance, so that generally the shaded side gets more auxin than the illuminated side, and consequently grows faster.

17. The sensitivity of cells for growth substance is diminished by light, so that in the dark the same quantity of auxin causes a greater elongation of the cell than in the light; this is known as the photo-growth response.

18. These effects of light are more especially due to light of short wavelength, red and orange light being ineffective.

19. Geotropism can also be explained by an unequal distribution of auxin.

20. When a negatively geotropic organ is placed in a horizontal position the quantity of auxin is greater in the lower than in the upper half, hence growth becomes faster on the lower side and a curvature ensues.

21. This uneven distribution disappears when such an organ is rotating on the horizontal axis of a klinostat.

22. During the transport of the growth substance, this is also deviated to the lower side of the organs when these are brought out of their normal position.

23. The fact that auxin inhibits the growth of roots gives an explanation of positive geotropic curvatures, when here too this unequal distribution of auxin takes place.

24. It is possible that the geoelectric effect discovered by Brauner may give an explanation of this unequal distribution of growth substance in organs which are kept in a horizontal position.

25. Autotropism may also be explained by the distribution of growth substance.

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THE DERMATOPHYTES

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CONTENTS.

I. INTRODUCTION.

THE dermatophytes are a group of fungi which parasitise man and animals by invading the keratinised layers of the epidermis and epidermal appendages such as hair, nails, hooves, horns and feathers. The diseases resulting from this parasitism, ringworm and favus, have attracted considerable attention in recent years. The whole field was reviewed from the mycological standpoint by Tate (1929a) in this journal. A number of important studies on different phases of the subject have since been published, and the present review of the recent literature is intended to indicate the main lines of research into the mycology of the dermatophytes, beginning approximately from where Tate's survey left off. Studies in the immunological and therapeutic aspects of the dermatomycoses are not included in the scope of this review. Questions of the synonymy of specific names are also excluded, on account of the confusion existing in the nomenclature of the group.

Among recent general reviews of this and other branches of medical mycology must be mentioned those of Bruhns and Alexander (1928), Bloch (1928a, b), Sabouraud (1928a, b, 1929a-d), Ramsbottom (1931) and Vuillemin (1931). The work of Catanei (1933) in Algeria, and of various Japanese investigators must be mentioned as examples of the local dermatophyte floras now appearing in many countries.

Sabouraud (1910) described 45 species of dermatophytes. The number of species named in the literature is now great; a cursory survey of the accessible literature made by the writer showed that at least 184 species have been recorded as causing disease in man. They were distributed according to Sabouraud's classification into the following genera: *Achorion*, 22; *Epidermophyton*, 19; *Microsporon*, 37; and *Trichophyton*, 106 species. The description of new species has progressed continuously, usually upon slight differences in the gross appearance of cultures on Sabouraud's standard media, rather than upon the morphology of microscopic organs. Davidson, Dowding and Buller (1932) introduced a new criterion of specific differentiation among the dermatophytes which in time may have the effect of disciplining the multitude of species to which ringworm is attributed. These writers regarded the fusions or anastomoses between the vegetative hyphae of the mycelium as significant. Working with hanging-drop cultures of *Microsporon audouini*, *M. lanosum* and *Trichophyton gypseum* they found that fusions were formed between hyphae of any two mycelia of the same species isolated from different patients. On the other hand hyphal fusions were not formed between mycelia of different species. The writers suggested that "the occurrence or non-occurrence of hyphal fusions between hyphae of two mycelia of different origin may be applied as a criterion for identifying species of dermatophytes whose specific nature is uncertain."

II. THE PARASITIC PHASE.
(1) *Dermatotropism.*

The dermatophytes have in common one physiological habit, that of growing in the horny layers of the skin. These fungi appear to possess the rare property of being able to invade and utilise the highly insoluble sclero-protein *keratin* which is present in cornified animal epidermal tissues. Tate, however (1929b), in his studies of the enzymes of dermatophytes was unable to extract a keratolytic enzyme from cultures. Such an enzyme still remains to be isolated from the dermatophytes. The ability to feed upon keratinised tissue is also known among such fungi as *Ctenomyces serratus*, *Onygena* spp. and probably species of *Aspergillus* and *Scopulariopsis*. Among insects it is known in the clothes moth, *Tinea*, the carpet beetles, *Dermestes*, and the biting lice, *Mallophaga*. In the crops of certain vultures, *Astur palumbarius* and *Vulturus monachus* predacious on other birds, Stankovic, Arnovljevic and Matavulj (1929) demonstrated a keratolytic enzyme which liberated amino acids from horny tissue. The occurrence of hair-balls in the stomachs of mammals and of fur in owl-pellets indicates the indigestibility of keratin to most vertebrates. The rather peculiar ability to grow on keratin constitutes the advantage which the dermatophytes have over most other fungi and forms the basis of their parasitic relationship with the host.

While there is no doubt that the dermatophytes are well adapted to parasitise the skin, the balance of evidence indicates that they are unable to parasitise other organs of the body. Some of the earlier experiments on this point which are summarised briefly by Fried and Segal (1929) appear inconclusive, but recent experiments indicate that the dermatophytes are unable to attack internal organs. Brocq-

Rousseu, Urbain and Barotte (1926) injected large doses of *Achorion* and *Microsporon* spores into guinea-pigs. Subsequent histological examination of the animals never revealed invasion of internal organs by the parasite, and they concluded that the dermatophytes only parasitise the skin. They then investigated the effect on the skin of inoculations into other parts of the body. Emulsions prepared from virulent cultures of *Trichophyton gypseum* and *T. equinum* were injected into the veins, into the peritoneum, under the skin, or fed through the mouth of guinea-pigs. Immediately after inoculation the back of each animal was scarified. In from 12 to 15 days two-thirds of the animals showed ringworm lesions, not at the site of the inoculation, but at the site of the scarification. The writers concluded that by whatever route the parasite is introduced into the body the only receptive organ is the skin. Cultures introduced into the body tend to become localised in the skin and to develop where the skin is damaged. Sulzberger (1929) injected guinea-pigs subcutaneously and intracardially with spore emulsions of *Achorion quinckeanum* but never obtained infection of internal organs.

This general principle receives confirmation by somewhat similar experiments on rabbits performed by Fried and Segal (1929). An area on the back of the animal was shaved and scarified, the animal was then wrapped in a blanket, and emulsions from cultures of *Trichophyton gypseum* were injected intravenously. The site of the insertion of the needle was then cauterised and painted with iodine; precautions were also subsequently taken to prevent external infection of the scarified area. Over a third of the animals used in the experiment developed ringworm on the scarified areas only. At intervals of from $1\frac{1}{4}$ to 72 hours after the injection, blood samples were taken aseptically and planted on media. The large number of cultures which were obtained from the blood indicated that the spores of the fungus which had been introduced intravenously continued to circulate in the blood for not less than 3 days. The scarification apparently injured the walls of the dermal capillaries and allowed the spores to reach the skin where the fungus could develop its pathogenic properties.

Dhayagude (1931) also concluded that the dermatophytes are only pathogenic towards the skin. However, he was unable to obtain a lesion on the scarified skin after subcutaneous or intraperitoneal injection of spores. He concluded that the positive results obtained by previous workers were due to an accidental contamination of the skin. It should be pointed out that Dhayagude did not exactly reproduce the technique of previous workers. Brocq-Rousseu made the scarification at the same time as the injection, while Dhayagude delayed scarification for a day or more.

Infection of the skin by certain species, such as *Microsporon audouini*, leads to a superficial form of ringworm. This parasitism consists in little more than the attack by the fungus on already dead and keratinised tissue. Infection by other species is usually accompanied by a varying degree of inflammatory reaction on the part of the body, a complication which makes it impossible to interpret the disease on purely parasitic concepts. The penetration of the epidermis by the fungal hyphae, and the reactions of the body to the invasion, are described by Sabouraud (1910), who makes the spatial relations between fungus and tissue the basis of his classification of the ringworm fungi.

(2) *Mycids and cultures from the blood.*

The classical concept of the pathological activity of dermatophytes, confirmed by daily clinical observation, is that of an active localised infection of the epidermis which may or may not be accompanied by inflammation of the surrounding tissues. Such a primary localised infection is called a *mycosis*.

Several distinct avenues of research, some dating from the beginning of the present century, are now seen to converge, indicating that the classical concept was incomplete and that ringworm is not always the purely local dermatophyte infection it was once thought to be.

The first enlargement of the concept came when Jadassohn (1912) discovered that primary dermatophyte infections are sometimes accompanied by secondary non-parasitic lesions on distant parts of the body. Jadassohn had under observation a number of children suffering from an inflammatory ringworm of the scalp belonging to the clinical type known as kerion. As the infection at the primary focus subsided, a peculiar rash consisting of groups of small red papules situated around the mouths of the hair follicles appeared on the body. In some cases each papule had a horny spine protruding from it. They soon faded away as the patient recovered. The peculiar characteristic of these lesions is that on microscopical examination the fungus was not found in them. As these secondary lesions were obviously not determined by the parasitic invasion of the dermatophyte concerned, Jadassohn explained the phenomenon as an action on the skin of toxins in spores which fell from the scalp lesion and were rubbed into the skin by the clothing. Further, he found that the secondary lesions could be reproduced by rubbing the fungus into the skin of other children with the disease. Subsequent evidence has tended to discountenance this view of the external origin of the secondary lesions. The rashes usually appear suddenly over wide areas and are symmetrical in their distribution on the right and left sides of the body. These facts suggest that the eruption is caused by fungus spores or toxins which are actually carried in the blood stream. Investigations on this problem will be considered later.

Since Jadassohn's original discovery in 1912, numerous observers have confirmed the association of a disseminated non-parasitic secondary lesion with a primary dermatophyte infection. The earlier literature is summarised by Low (1924).

The primary localised fungus infection is known as a *mycosis*, and the secondary lesion as a *mycid*. More specifically, a mycid may be a trichophytid, a microsporid, or an epidermophytid, according to the species responsible for the primary lesion.

In addition to the generalised papular rash previously mentioned, a variety of mycid eruptions are now known to be associated with different types of dermatophyte infection. Thus C. M. Williams (1927) and others have shown that ringworm of the feet may be associated with squamous and vesicular lesions on the hands from which the parasite is absent. Muende (1930) reported a case of conjunctivitis as a trichophytid manifestation. C. M. Williams (1930, 1931) reported mycid eruptions as complicating ringworm of the groin and ringworm of the upper lip.

Peck (1930) proved the connection between ringworm of the feet and mycid eruption of the hands by an experimental inoculation. A volunteer was inoculated between the toes with an *Epidermophyton*, and typical ringworm developed from which the fungus was isolated. Twenty-four days after inoculation a vesicular eruption developed on the hands. This was apparently an epidermophytid, as the lesions on the hands were free from fungi. After spontaneous healing had occurred the feet of the patient were reinfected, and a second epidermophytid appeared two weeks after. Peck was apparently the first to produce a mycid experimentally in a previously uninfected human being.

That the more inflammatory dermatophyte infections are not merely local incidents is also shown by the fact that during the course of the disease the skin on distant parts of the body may become altered in its reaction and may show increased sensitivity to an injection of the fungus protein. A small quantity of trichophyton protein extract (trichophytin) injected into the skin of a normal individual usually results in little more than a transient inflammation. However, when injected into the skin of a patient suffering from one of the more severe kinds of ringworm, a marked inflammatory reaction, which may persist for several days, is produced at the site of the inoculation. The experimental and clinical aspects of this alteration in the reaction of the body, known as *allergy*, are beyond the scope of this review, but are summarised by Low (1924) and Bloch (1928 a). The subject is merely introduced here in order to emphasise the change in attitude which has taken place towards these fungi which were at one time considered to be merely local parasites.

The explanation now commonly given of mycids is that spores or toxins liberated by the fungus in the primary lesion pass into the blood stream and circulate there, thus reaching the skin which, as a result of the infection, has become allergic or hypersensitive to the presence of the protein. The reaction of the skin to their presence constitutes the mycid. At first sight it might appear more probable that the substances liberated by the fungus in the primary lesion would be soluble toxins. Thus Bloch (1928 b) points out that a mycid eruption may follow an injection into a patient of a protein extract from a culture of the fungus. On the other hand, there is a certain amount of evidence to indicate that the transported substances are fungus cells. The spores which form the sheath around *Trichophyton* infected hairs vary according to the species but are not too large to circulate in the blood stream except in the finest capillaries. The diameter of a human red blood cell is about $7\cdot5\mu$, while the spores of the *Trichophyton microides* group are about 3μ, and those of the *T. megalosporon* group about 6μ in diameter.

The transport of fungus spores by the blood is not merely hypothetical, as there is an accumulation of records of actual isolations of dermatophytes from aseptically drawn blood samples of patients with various dermatomycoses. The earlier records were of species of *Trichophyton*. Thus, Sutter (1920) isolated *T. granulosum* from the blood of a patient with scalp ringworm; Ambrosoli (1921) isolated *T. gypseum* from the blood of a patient with kerion; Jessner (1921) isolated *T. granulosum* from a patient with scalp ringworm; Pasini (1921) isolated a *Trichophyton* sp. from a ringworm patient. *Microsporon audouini* was isolated from the

blood by Arzt and Fuhs (1923) and by Schmidt (1933). *Achorion schoenleini* was isolated from the blood of patients with favus first by Ambrosoli (1926) and later by Lourier and Rieff (1932). Cultures have also been obtained from the blood of patients with ringworm of the feet. Peck (1929 and 1930) isolated an *Epidermophyton* from the blood of a patient with a mycotic infection of the feet associated with a mycid eruption on the hands. Three other isolations of fungi from the blood of patients with mycotic diseases of the feet were noted by Strickler, Ozellers and Zaletel (1932). Earlier than either of these two records is the case reported by White (1928) in which ringworm of the feet due to *Trichophyton interdigitale* was associated with enlargement of the inguinal lymph nodes. Material was aspirated from one of the glands by means of a sterile syringe and when planted on Sabouraud's dextrose agar gave a culture of the *Trichophyton*. A gland was subsequently excised and both mycelium and spores were observed microscopically in portions macerated in potash. Evidently the fungus had reached the lymphatic glands from the superficial primary focus in the feet. Records of isolations from the blood are summarised in Table I.

Table I. *Isolations of dermatophytes from the circulating blood.*

Author reference	Fungus
Ambrosoli, 1921	*Trichophyton gypseum*
Ambrosoli, 1926	*Achorion schoenleini*
Arzt and Fuhs, 1923	*Microsporon audouini*
Fried and Segal, 1929 (from inoculated rabbits)	*Trichophyton gypseum*
Jessner, 1921	*Trichophyton granulosum*
Lourier and Rieff, 1932	*Achorion schoenleini*
Masia, 1924	*Trichophyton cerebriforme*
Pasini, 1921	*Trichophyton* sp.
Peck, 1929, 1930	*Epidermophyton* Kaufman-Wolf
Schmidt, 1933	*Microsporon audouini*
Strickler *et al.*, 1932	*Epidermophyton* (?)
Sulzberger, 1928 (from inoculated guinea-pigs)	*Achorion quinckeanum*
Sutter, 1919–20	*Trichophyton granulosum*
White, 1928 (from lymph nodes)	*Trichophyton interdigitale*

With artificially inoculated guinea-pigs even more striking results have been obtained by Sulzberger (1928) working in the clinic of the late Prof. Bruno Bloch at Zurich. Over one hundred guinea-pigs were infected with *Achorion quinckeanum* by rubbing a sporulating culture into the skin of the back after slight abrasion with sand-paper. From these animals 140 blood samples were removed aseptically from the heart and planted on culture media. After incubation sixteen of these blood samples gave cultures of the organism inoculated. Cultures of the fungus were obtained from samples of the blood taken during the first two days after inoculation. Then came a negative phase, and no more cultures were obtained until the tenth to thirteenth day. This second later phase in which fungi were again present in the blood corresponded with the acme of the inflammatory process in the primary lesion, and with the beginning of the rapid healing which the primary lesion underwent.

Sulzberger suggested on the basis of these experiments that in man also mycids might be explained by the dissemination through the blood stream of fungus elements liberated from the primary lesion shortly before the appearance of the mycid eruption.

The widening concept of the activity of the dermatophytes here presented is a crystallisation out from several lines of investigation: clinical records of mycid eruptions; experiments on allergy or the development of hypersensitivity to fungus proteins; experiments on the localisation of dermatophytes to the skin; and evidence derived from isolations of various dermatophytes from the blood by a number of workers in different countries.

(3) *The fluorescence of infected hairs.*

An empirical weapon of considerable value was placed in the hands of the mycologist by the discovery that hairs infected by certain species of dermatophytes fluoresce in ultra-violet light.

Patients suffering from various skin diseases were examined by Margarot and Devèze (1925) in a darkened room by means of the so-called Wood's light. This is the radiation from a source of ultra-violet light passing through a filter of Wood's nickel oxide glass. This filter is almost opaque to visible light, but relatively transparent to the longer wave ultra-violet radiation lying just outside the visible spectrum. Margarot and Devèze observed that hairs from cases of microsporon ringworm or from favus shone with a greenish fluorescence entirely different from the bluish tint of the normal skin. They stated that cultures of these fungi on Sabouraud's medium were also fluorescent. The filtered ultra-violet light made it possible to reveal small foci of infection on the hairy skin as small brilliant fluorescent patches which it was sometimes impossible to demonstrate by ordinary light.

The phenomenon appears to be a fluorescence rather than a phosphorescence, as the emission ceases as soon as the excitation by ultra-violet light is discontinued. Further, the phenomenon follows Stokes' Law, as the emitted green radiation is longer in wave-length than the incident ultra-violet radiation. The fluorescence test is now widely used in clinical examination of patients and contacts, in following the progress of treatment and in detecting carriers.

Vigne (1927) gave a careful and detailed description of the phenomenon based on a study of a large number of cases of scalp ringworm with ultra-violet light. The diagnoses were confirmed by microscopic examination and by cultures on Sabouraud's media. He demonstrated that fluorescent hairs were always infected and that non-fluorescent hairs were not infected. The fluorescence of ringworm hairs due to a *Trichophyton* depended on the species present, but was always a bluish green, not a brilliant green as in *Microsporon* ringworm. Observations on favus showed that infected hairs emitted a green fluorescence, but that the characteristic scutula of favus were not fluorescent. Cultures of dermatophytes were found to show a violet fluorescence, which tended to appear yellow in old dried up tubes. Vigne concluded that the fluorescence of ringworm hairs is due to the presence of spores of the fungus, and he was not surprised to observed that hairs infected by an endothrix

Trichophyton are less luminous than those infected by a *Microsporon* where numerous spores are situated around the outside of the hair.

In a later paper by Margarot and Devèze (1929) the phenomenon is similarly explained as being due to the fluorescence of the fungus itself. "La fluorescence propre des divers agents des teignes explique la teinte verdâtre observée en pareil cas. Les recherches de Vigne et les nôtres établissent que la luminosité est due au champignon lui-même. Nous avons pu nous rendre compte que dans le monde végétale d'autres champignons microscopiques étaient fluorescents. En particulier, les taches de mildiou de la vigne ont une luminosité verte et très comparable à celle des microspories."

Kinnear (1931) also regarded the green fluorescence as due to the fungus in cases of infection by *Microsporon audouini*. He observed a paler fluorescence in hairs infected by *Trichophyton crateriforme*, *T. acuminatum*, *T. sulfureum*, and *T. polygonum*, but in ringworm caused by these endothrix trichophyta and also in favus he attributed the luminosity to the fluorescent properties of the keratin of the hair. He stated that microscopic examination of hairs infected by *Microsporon* showed that the fungus itself was fluorescent, and also that the fluorescence was retained indefinitely when the hairs were placed in potash.

Davidson and Gregory (1932) confirmed the results of the previous workers who showed that the presence or absence of green fluorescence is determined by the species of fungus present. Green fluorescence was observed in hairs infected by *Microsporon audouini*, *M. felineum* and *Achorion schoenleini*. On the other hand in hairs infected by *Trichophyton album*, *T. gypseum* and *T. violaceum* a faint to bright bluish luminosity was noticed. Vigne (1927) and Margarot and Devèze (1929) believed that the fluorescence observed was that of the fungus itself, and attributed the faintness of the luminosity of the endothrix trichophyta to the obscuring of the fluorescence by the hair-keratin surrounding hyphae and spores. Davidson and Gregory (1932) questioned this explanation on the ground that hairs infected by the ectothrix species, *T. gypseum* and *T. album* do not show green fluorescence, while favus hairs, in which the hyphae occupy an endothrix position, fluoresce with a green light. They considered that the fluorescence was situated in the hair rather than in the fungus. Transverse sections of hairs infected by *Microsporon audouini* showed that the green fluorescence resided principally in the shaft of the hair, while the external spore sheath appeared bluish. They suggested that the fluorescence is due to some change in the hair substance following invasion by the fungus, perhaps a product of hydrolysis of the keratin. Further, they discovered that by treating hairs infected by *M. audouini*, *M. felineum*, or *Achorion schoenleini* with potash, or with warm water after defatting in ether, a fluorescent substance could be extracted in the form of a fluorescent aqueous solution, leaving the hairs non-fluorescent. Such a substance could not be extracted from normal or from *Trichophyton*-infected hairs. The chemical nature of the fluorescent substance has yet to be studied.

III. THE SAPROPHYTIC PHASE.

(1) *The question of natural saprophytism.*

The fact that the dermatophytes will live saprophytically in culture has long been known. Sabouraud (1910) introduced two principal media for the cultivation of the dermatophytes (see Tate, 1929 *a*). His *milieu d'épreuve* is a maltose peptone agar and is an attempt to produce a synthetic medium chemically resembling beer-wort, on which these fungi were known to grow luxuriantly. The medium is recommended for making isolations from infected material, and also for identifying Sabouraud's several species of dermatophytes which are based on differences in the gross appearance of colonies on this medium. Cultivation over a long period on this maltose peptone medium induces, in most species of dermatophytes, a form of degeneration known to medical mycologists as "pleomorphism." Sabouraud therefore introduced a *milieu de conservation*, a peptone agar which, while not bringing out the differential characteristics of all species, has the property of postponing the pleomorphic change indefinitely. Despite their proven practical utility these media fall short of the ideal standard synthetic medium. If mycelia comparable with those depicted by Sabouraud are to be obtained, the media must be compounded of maltose *brute de Chanut* and of peptone *granulée de Chassaing*, procurable only through one firm in Paris. However, these media have become the standard, and the morphological classifications of the dermatophytes proposed by Ota and Langeron (1923) and by Grigoraki (1924, 1925) are based on microscopic studies of cultures on these media alone.

The war led to irregularities in the supply of the maltose required for the preparation of Sabouraud's "proof medium." In America this resulted in attempts to find other brands of sugar and peptone which would replace the French products while giving cultures of the same gross appearance as the true proof medium. Although progress in this direction was reported by Weidman and McMillan (1921), Weidman and Spring (1928), and Hodges (1928), the problem has not been solved. European workers proposed entirely new media which still suffer from the defects of comprising proprietary ingredients (*e.g.* Grutz, 1923; Goldschmidt, 1924). Sabouraud (1925) used honey as a substitute for maltose, and later Sabouraud and Negroni (1929) showed that this medium particularly favoured the production of aleuriospores (conidia) by *Achorion schoenleini*. It cannot be claimed at present that any of the newer media have replaced Sabouraud's original formulae by providing good differentiation on a medium of constant known composition. However, the investigation has served to demonstrate that dermatophytes can be grown satisfactorily on many media besides those devised by Sabouraud. This fact was generally ignored, although Sabouraud (1910, p. 726) had discovered that these fungi would grow easily on such substances as cereals and decayed wood. He therefore suggested that they may exist in a natural saprophytic state and that for many species the parasitic existence may be only occasional. It is in following out this idea that the most interesting developments have taken place.

While parasitic on the skin the dermatophyte thallus is differentiated only into

hyphae and small round thallospores or oidia. On artificial media many dermato-phytes may produce a wealth of organs which are unknown outside the laboratory. This circumstance has led many workers to suspect that these fungi can go through an unknown saprophytic stage in nature in which such highly differentiated struc-tures as aleuriospores, fuseaux, spirals and other organs, play a part. Such a sapro-phytic phase has a practical interest as a possible source of infection for animals.

Nannizzi (1926, 1927; see Tate, 1929 *a*) considered the morphology of the derma-tophytes on synthetic media to be teratological. He believed the natural saprophytic habitat of these fungi to be animal decidua such as hair, feathers, skin and leather, on which substances under experimental conditions he cultivated various species satisfactorily. The use of sterilised hair as a culture medium was also proposed by J. W. Williams (1934), who suggested that ability to grow on hair might be used as a means of differentiating dermatophytes from other fungi.

The hypothesis that vegetable rather than animal substrata form the natural saprophytic environment of the dermatophytes has led to an interesting series of studies by French workers. Brocq-Rousseu, Urbain and Barotte (1928 *a*, *b*) found that a strain of *Trichophyton gypseum* from a horse grew abundantly on substances such as grains of cereals, hair, dung and debris of horn present in the litter and fodder of domestic animals. They found that cultures on these substrata remained viable for a considerable time. *T. gypseum* on a blade of straw was viable after two and a quarter years, while *Microsporon equinum* survived for over three years. By means of inoculations they were able to show that such cultures retained their virulence for animals. They considered that these facts supported the hypothesis that animal ringworm may be conveyed in fodder. Catanei (1928) confirmed this work by growing *Trichophyton radiolatum* on sterilised fragments of straw, in moist tubes. The culture which developed at room temperature proved pathogenic when re-inoculated into guinea-pigs. Urbain (1928) grew *T. gypseum* for several months on the litter at the bottom of guinea-pig cages. Animals placed in the cages after scari-fication of the skin became infected with the fungus. Bonar and Dreyer (1932) cultivated *T. interdigitale* successfully on weathered wood and on floor material that was covered by a coating of slime and algal growth.

The foregoing investigations established the ability of dermatophytes to grow on a variety of vegetable substrata. Investigators had, however, apparently been content to evaluate the suitability of these natural media for dermatophytes by judging the amount of mycelium visible to the naked eye. Little was known of the organs produced on such media until Langeron and Milochevitch (1930 *a*, *b*) took up the subject at the point where Brocq-Rousseu and his collaborators left off. Nan-nizzi had previously shown that dermatophytes grew saprophytically on animal substrata, but Langeron and Milochevitch considered that the more abundant vegetable substrata are the natural reservoirs of infection by the ringworm fungi. They cultivated twenty-five species of dermatophytes, including many of Sabour-aud's type cultures, on a variety of vegetable substances. Their media were of two types: (1) natural substances such as grains of wheat, barley and oats, straw, horse dung, mushrooms; and (2) synthetic media in which polysaccharides predominated,

including starch, dextrin and wheat flour agars. Expecting the structural development to be poor or rudimentary, they were surprised to find a rich microscopic morphology in which the various organs, aleuriospores, fuseaux, spirals, etc., were as well or even better developed than on Sabouraud's classical media. Some species produced organs which were unknown in them before; thus *Microsporon felineum* on oats produced spirals resembling those which characterise certain trichophyta. A further effect of these media in suppressing pleomorphism will be dealt with below.

Davidson and Gregory (1933, 1934) suggested that the saprophytic type of spore might be found in nature in moist places on infected tissue which had been shed by an infected animal in the normal course of the disease. They pointed out that the dermatophytes are relatively slow-growing organisms and apparently less well adapted to colonise dead vegetable matter than most of the common saprophytes, but that when growing on keratinised tissues, which few other organisms are able to attack, they are free from competition. Naturally infected hairs, in which the fungus showed the rudimentary structure of the parasitic phase, were removed from patients suffering from ringworm due to *Microsporon audouini*, *M. felineum* and *Trichophyton gypseum*. The specimens were attached to the cover glasses of van Tieghem cells containing moist atmospheres controlled by osmotic solutions of known concentration. The fungus was thus supplied with water vapour, but no nutrient medium of any kind was added. The fungus which had parasitised the hair soon entered on a second phase of growth in which all the organs characteristic of cultures on artificial media were developed to perfection. Scutula from cases of favus due to *Achorion schoenleini* also germinated in a moist atmosphere and produced numerous aleuriospores (conidia). The authors suggested that animals with ringworm may often shed infected hairs and scales in moist places. Under suitable conditions the parasite may resume growth as a saprophyte on the detached animal tissue. In this new phase it may produce the organs characteristic of its saprophytic phase, as it did under experimental conditions.

Thus laboratory evidence supports the hypothesis that either an animal or a vegetable substratum may serve for the saprophytic stage. Not until fungi recognisable as dermatophytes on grounds of structure and inoculability have been collected and isolated from field material will the problem of the saprophytic phase be elucidated. Demonstration now rests with the outdoor mycologist.

(2) *Pleomorphism and variation.*

Grigoraki (1929, 1933) in a series of papers contended that the dermatophytes show neither true polymorphism nor pleomorphism. He considered that the changes occurring in strains kept in culture over long periods on media containing sugar represent merely a gradual degeneration towards a monomorphism in which the morphology of the various species converges to a form consisting of narrow, sterile hyphae.

Sabouraud (1929c), on the other hand, reiterated his clear distinction between changes due to senility and those due to pleomorphism. Senility occurs in aged cultures, and these can be rejuvenated when transferred to fresh medium. Pleo-

morphism is a definitive morphological change occurring in old cultures of certain species; it is perpetuated in transfers on fresh medium. It consists in the appearance on mature cultures of a white downy mycelium which can be isolated from the parent and which does not revert to the parent sporulating form even when inoculated into animals and reisolated. "Il s'agit donc ici, non pas d'un phénomène transitoire, mais d'une *mutation fixe*."

The pronounced difference between the normal and pleomorphic form is illustrated by an experiment reported by Langeron and Tallice (1930). Guinea-pigs were inoculated with pleomorphic strains of *Sabouraudites felineus* [*Microsporon felineum*]. The normal structure of this fungus in the parasitic phase consists of hyphae within the hair and an ectothrix sheath of spores. The pleomorphic strains, however, attacked the guinea-pig hairs as an endothrix form which showed no trace of sporulation.

The researches of Sabouraud had shown that the pleomorphic change took place on media containing sugar, and that its occurrence could be avoided by cultivation on a peptone medium free from sugar. On their new natural media Langeron and Milochevitch (1930 a) grew four of Sabouraud's strains which readily become pleomorphic on sugary media: *Sabouraudites* [*Trichophyton*] *asteroides*; *S.* [*T.*] *granulosus*; *S.* [*T.*] *lacticolor*; and *S.* [*Achorion*] *gypseus*. On grains of cereals, straw, dung, and on synthetic media containing soluble starch, dextrin or wheat flour, these strains showed no signs of pleomorphism. Langeron and Milochevitch (1930 b) concluded that the simpler molecules of monosaccharides and disaccharides, such as dextrose and maltose, are toxic to the fungus and provoke the pleomorphic change, and that, like many other living organisms, the dermatophytes need to break down large complex colloidal molecules such as they find in natural media and in media prepared from polysaccharides.

A study of variations occurring in a culture over a number of years was made by Biltris (1929). Isolations on Sabouraud's medium from a mouse with ringworm gave strains of two types. One had white powdery colonies, and was apparently a strain of *Trichophyton gypseum asteroides*. The other was a reddish slower growing colony with moist areas resembling young yeast colonies. This form showed numerous sectors and gave rise to a series of variations whose changes were followed over a number of transfers to fresh media. Some of the more stable forms which arose were submitted to Dr Sabouraud and were said to resemble such widely differing species as *Microsporon equinum*, *Trichophyton gypseum asteroides*, *T. gypseum granulosum*, and *T. lacticolor*; another resembled *T. crateriforme* or *T. plicatile*, and one was a new species. In view of these transformations of a single primary culture Biltris concluded that the doctrine of the plurality of species of dermatophytes does not fit the facts and that classifications based on this doctrine are superfluous. The validity of this sweeping conclusion is, however, shaken when the writer mentions incidentally that the red slow-growing parent colony of the series was contaminated by a red *Torula*. The subsequent sectors and variants which arose may well have been merely stages in the sorting out of the two organisms.

Emmons (1931) designed experiments to test Grigoraki's (1929) hypothesis that

the aleuriospores (conidia) represent degenerate forms of the fuseaux (macroconidia), and that the downy or pleomorphic type of culture might arise from only one of the spore types present. Emmons studied cultures of *Achorion gypseum* Bodin, a fungus which had the advantage of producing abundantly both small aleuriospores and fuseaux. A number of single spore isolations were made, starting with both types of spore. Cultures from either aleuriospores or fuseaux gave nearly identical mycelia, the only observable difference being that the initial colonies from fuseaux were slightly larger than those from aleuriospores, perhaps a result of the greater supply of food stored in the larger spores. In subcultures from the two forms this difference was lost. Microscopically, cultures from either source were identical, and there was the same proportionate occurrence of the two spore forms in both. Further, monospore cultures from either source became pleomorphic at about the same time. The pleomorphic mycelium produced many aleuriospores and a few small fuseaux. Monospore cultures from these two types of spores yielded exactly similar pleomorphic mycelia. These results were confirmed by a similar series of experiments on *Trichophyton gypseum*. Emmons concluded that there is no evidence that aleuriospores and fuseaux which are formed simultaneously are genetically different. "The conidia formed in pleomorphic cultures do, however, differ from those formed in the original type of culture. Just how and where this change takes place is not yet clear."

In a later paper Emmons (1932) described a number of variants which suddenly appeared in a culture of *Achorion gypseum* growing on horn. The parent strain from which these variants arose was descended from a single aleuriospore which was presumably uninucleate. Ninety-six monospore cultures were made from the variants, which whether from aleuriospores or fuseaux gave cultures reproducing the variant from which they originated. In some cases the variants in turn gave rise to new varieties. While old cultures of the parent strain of *A. gypseum* readily became pleomorphic, it is noteworthy that none of the variants underwent this change. However, when in some cases the variants reverted to the parent form, they again became subject to the pleomorphic change. Instead of arising in the form of sectors as is frequently the case with fungal mutations, these variations arose as tufts of abnormal growth on the parent culture. The pleomorphic change also arises in the same way.

In the light of recent work it appears that pleomorphism is another example of the irrevocable mutation or saltation so common in many fungi, rather than a gradual process of degeneration on artificial media. It is also clear that pleomorphism is only one type of mutation to which the dermatophytes are liable.

(3) *Asci and heterothallism.*

The dermatophytes normally reproduce asexually by means of chlamydospores or conidia, both in the parasitic condition and in culture. As a sexual stage does not habitually occur in the life history they are logically classified among the Fungi Imperfecti. This is a heterogeneous group of forms in which sexual stages, which form the normal basis of fungus classification, are unknown. It is continually being

discovered that some imperfect fungus is merely the conidial stage of some ascomycete, and that suitable treatment will induce it to form asci. Attempts have therefore been made to connect the dermatophytes with some of the higher fungi, and several workers have now reported the occurrence of asci. Such a discovery, if corroborated, would naturally mark a great advance in the study of these organisms. The ascus stage may give a clue to the saprophytic existence of these fungi. It would also be of great interest to students of the variability of morphology and virulence, as it is in the ascus that reduction division and the sorting out of hereditary factors normally take place.

The earlier records of an ascus stage in the dermatophytes, which although dealt with by Tate (1929 a) are here enumerated for the sake of completeness, are those of Matruchot and Dassonville (1901) in *Eidamella spinosa*; Chalmers and Marshall (1914) in *Trichophyton currii*; and Nannizzi (1927) in *Gymnoascus* (*Achorion*) *gypseus*. Tate (1929 a) and Emmons (1931) were unable to repeat Nannizzi's results, and all three of the earlier records were adversely criticised by Langeron and Milochevitch (1930 b). *Eidamella spinosa* (isolated from a dog) undoubtedly produced asci, but Matruchot and Dassonville did not establish its pathogenicity. The asci of *Trichophyton currii* are considered to be those of a contaminant belonging to the Perisporiaceae, such as an *Aspergillus*. Nannizzi is criticised on the grounds that his cultures were grown on unsterilised media. Criticisms of Nannizzi's work were, however, replied to by Pollacci (1931), who refuted the assertion that the results were obtained from impure cultures, and concluded that Nannizzi was the first to demonstrate the position of the dermatophytes in the Gymnoascaceae.

Wilenczyk published a series of observations which he interpreted as evidence of ascus formation under special conditions. In his first reports, Wilenczyk (1928 a, b) claimed to have observed immature asci in hanging-drop cultures growing at body temperature. The organs he observed appear, however, to have been merely the knots of hyphae named nodular organs by Sabouraud. Wilenczyk clearly did not appreciate the true nature of asci or perithecia. Wilenczyk (1927, 1928 a) cultivated recently isolated strains of *Tricophyton granulosum* and *T. violaceum* on Sabouraud's proof medium at 37° C., transferring every two or three weeks to fresh medium. After six transfers they were grown in hanging drops of sugar bouillon at 37° C. After five or six days these cultures were removed to room temperature, and oval or elongated yellow sacs developed containing 2, 4, 6 or 8 separate corpuscles. These bodies were interpreted as spores within an ascus. When the asci were transferred to a fresh drop the envelope disappeared in 24 hours and each spore germinated. The organs could also be obtained on proof medium by making a series of transfers at 37° C. and then bringing the cultures to room temperature. In *T. violaceum* the bodies were said to be sufficiently abundant to colour the colony brown or black. Cultivation at a high temperature followed by growth at room temperature was considered to induce ascus formation. Oxygen was also a favourable factor, as when the flasks were plugged too tightly asci were not formed. An examination of the figures which accompany the paper suggests an entirely different interpretation. The so-called "mature asci" are apparently dark multiseptate conidia belonging to some member of the Dematiaceae-phragmosporeae group of the Fungi Imperfecti,

such as an *Alternaria*. The figure of a germinating "ascus" shows that the supposed ascospores had not separated from one another, and again suggests that the author mistook the individual cells of the septate conidium of a contaminant for ascospores. The fact that the culture became black confirms the hypothesis of an *Alternaria*-like contaminant, as also does the fact that tightly plugged flasks did not show the phenomenon. The hanging-drop culture technique used by Wilenczyk (1926a) is open to criticism. The results obtained can most simply be explained by supposing that the cultures studied were contaminated with a black mould which sporulated at room temperature but not at 37° C. Wilenczyk (1928b, 1929b) reported that *Achorion schoenleini* and *Epidermophyton* formed asci identical with those of *Trichophyton*. These "asci" were later (Wilenczyk, 1928c) discovered in scales taken from patients with favus and trichophytosis, and also in scales from patients with seborrhoea which were examined as controls. Mature "asci" obtained by drying portions of cultures of *T. granulosum*, *T. equinum*, and *Achorion schoenleini* were figured by Wilenczyk (1929a) and only serve to confirm the supposition that the writer was dealing with a contaminant, especially as Wilenczyk (1929c) found difficulty in inoculating animals with ascogenous cultures, or in reverting the black cultures to the original form.

Another investigator who reported asci was Biltris (1929). In his cultures, previously referred to, derived from a lesion on a mouse, ascus-like structures were observed. Some of his figures indeed suggest flask-shaped asci containing ascospores, but most of them may be equally well interpreted as chlamydospores. The "asci" are shaped much as the chlamydospores seen in old cultures of the dermatophytes, and the round bodies within them vary in size and number in a manner suggesting fat droplets rather than spores. These supposed spores were not germinated. Further, as noted above, the parent culture of Biltris's strain was admittedly contaminated by a red *Torula*.

Kambayashi (1932) described the occurrence of "askusähnlichen Organen" in aged liquid cultures of *Microsporon japonicum*. Here again, as in the observations of Biltris, it is possible that the organs observed were merely chlamydospores. The bodies within the cell varied greatly in size and number, and were not germinated. However, preparations stained with iron haematoxylin showed that the nuclei tended to be arranged in multiples of eight. Kambayashi therefore suggested that the flask-shaped structures represented either asci or compound asci according to the number of spores. A further communication promised on this subject will be awaited with interest.

Asci contained in perithecia were reported by Datta (1932) for a new fungus named *Eidamella actoni*. The fungus was isolated from lesions on the skin of a dog and gave typical lesions when inoculated into healthy dogs. The cultures were apparently those of a typical member of the Gymnoascaceae. While not identifiable with any known species, the organism resembled a dermatophyte both in habit and in certain morphological characters. The presence of septate spines and of pectinate organs indicated the genus *Eidamella*. Each of the numerous round asci contained more than eight ascospores.

It is not inherently improbable that asci will be found in the dermatophytes, but the contradictory nature of some of the present reports forces caution on the reader. It is, however, satisfactory that the results of Matruchot and Dassonville, Nannizzi and of Datta agree in relating the dermatophytes to the Gymnoascaceae.

The usual absence of asci from dermatophyte cultures can be explained in three ways. Some environmental factor such as a particular food substance, or conditions of temperature, humidity, etc. may require adjusting before the culture can form asci. Again, the sexual mode of reproduction may have fallen into disuse and become eliminated as a negative adaptation to parasitism. Or again it is possible that the dermatophytes are heterothallic. That is to say that there must be a union between two mycelia of opposite sign or sex before asci are produced. The foregoing investigators have tacitly assumed that the dermatophytes are homothallic and that the production of asci can be determined by controlling the environment. The possibility of heterothallism in the dermatophytes was suggested by Weidman (1929) and a study of the subject was subsequently made by Spring (1931). Ten single cell cultures from aleuriospores were made from each of two strains of *Trichophyton interdigitale* and from one strain of *T. purpureum*. The ten monospore isolates from each parent strain were paired in all possible combinations on peptone agar, and the colonies resulting from this cross planting were compared with colonies derived from the single spores. In addition to examining the cross plantings for the presence of asci, attention was paid to other changes which might have been induced by pairing the mycelia, such as pleomorphism and gross appearance of the colony. In none of the cross plantings made were any asci found, but differences in the pleomorphism and configuration of certain colonies were noted, which it was suggested might conceivably be expressions of heterothallism. Spring concluded that in the three strains tested heterothallism could not be distinguished with finality. The investigation is open to criticism on the ground that it was not planned to answer the question asked. If a fungus does not produce asci because it is a heterothallic species, then presumably the culture is a haploid, and is either (+) or (−) in sign. No amount of mating monospore isolations derived from the same haploid parent is likely to yield a diploid mycelium. A more profitable line of approach would be to collect a large number of these presumably haploid strains from different sources, and to pair them in all possible combinations in the hope that some of the haploid isolates might chance to be of opposite sign. Such a technique in the hands of Drayton (1934) has yielded valuable results with a plant parasite, *Sclerotinia gladioli*. Apparently Spring did not attempt matings between strains of different origin. A start has been made along these lines in the present writer's laboratory with isolates of *Trichophyton gypseum*. No success has yet been achieved, but the number of isolates available is small.

IV. AFFINITIES WITH OTHER FUNGI.

The dermatophytes first attracted attention as a physiological group having in common the habit of parasitising the keratinised epidermis. In spite of structural differences between different species, they appear to be more closely related to one

another than to other fungi. The only conidial stage known is of the aleuriospore type, that is to say the spores are not abstricted off from a sterigma like the conidia of a *Penicillium*, but are separated from the parent hypha by a septum, and shed on the disintegration of the hypha. In an increasing number of species fuseaux are becoming known, and in addition many species are now known to produce spiral hyphae. Serological reactions in general are not specific, and indicate a close relationship between the species. These facts are considered to justify the inclusion of the dermatophytes in a single family.

The affinities of the family have been a source of difficulty to mycologists. The dermatophytes have never been brought satisfactorily within any general system of fungus classification on purely morphological grounds. They have therefore always stood as a group apart, usually neglected by botanists.

Clements and Shear (1931) evade the difficulty in their key to the families of the Fungi Imperfecti by introducing a physiological character. The key proceeds on morphological criteria until suddenly the reader has to decide between:

A. Conidia present ....(etc.)
B. Conidia lacking ....(etc.)
C. Conidia present but criteria indefinite: parasites on human skin...*Dermophyta*.

In the section on saprophytism it was pointed out that no dermatophyte has been found occurring in nature except as a parasite. It is obvious that until the classification can be amended to include the dermatophytes on morphological grounds no botanist who is not already intimate with their structure will be able to identify, for example, a soil fungus as a dermatophyte.

Vuillemin (1931), believing that classifications based on characters other than those of sexual reproductive organs are of little value, distributed the dermatophytes among the various unrelated genera of the Fungi Imperfecti, such as *Mycoderma* and *Fusoma* (*vide infra*). Unfortunately Vuillemin never completed his interesting system of classification of the Imperfecti, and, while including most genera of medical interest, many saprophytes and plant parasites were not incorporated.

Affinities with the Gymnoascaceae have been assumed by Matruchot and Dassonville (1901), Nannizzi (1927), and Datta (1932), on account of their observations of the ascogenous stage. Langeron and Milochevitch (1930b) reach a similar conclusion from their discovery of so-called "sterile perithecia" in species of *Trichophyton*. Volkonsky (1934), in biochemical studies of various fungi, concluded that the dermatophytes are related to *Ctenomyces serratus*, a saprophytic member of the Gymnoascaceae, by their fermentation formula, their non-assimilation of nitrates and by their ability to assimilate sulphates. However, as only five other imperfect fungi were investigated, this similarity need not be taken to indicate a very close relationship.

An entirely different position was maintained by Biltris (1929) who considered that the dermatophytes were related to the Ascocorticiaceae. This hypothesis rested on the discovery of organs in his cultures which he regarded as naked asci, not enclosed in a perithecium. In the absence of definite evidence that the structures observed by Biltris were indeed asci of a *Trichophyton* this hypothesis is unsupported.

V. CLASSIFICATION OF THE DERMATOPHYTES.

The history of classification in recent years is one of revision of existing systems. Grigoraki (1924) had previously discarded the generic names of Sabouraud's system, *Microsporum*, *Trichophyton* and *Achorion*, and had introduced a totally new series of generic names. In his revised system Grigoraki (1929) reinstated Sabouraud's generic names in order to preserve priority according to the rules for botanical nomenclature. On the other hand he retained his own generic names as subgenera.

Grigoraki's revised classification (1929).

A. Genus **Microsporum**: primary cultures powdery owing to presence of fuseaux.

(*a*) Subgenus *Closterosporia*: with many fuseaux. Type species: *M. lanosum*.

(*b*) Subgenus *Closteraleurosporia*: very slightly powdery, rapidly becoming downy. Type species: *M. audouini*.

B. Genus **Trichophyton**: species with a downy culture, and with chlamydospores and aleuriospores in the primary culture. Type species: *T. crateriforme*.

(*a*) Subgenus *Chlamydoaleurosporia*: primary culture with large chlamydospores, or small chlamydospores and aleuriospores. Type species: *T. crateriforme*.

(*b*) Subgenus *Aleurosporia*: downy culture with aleuriospores. Type species: *T. acuminatum*.

C. Genus **Achorion**: species with glabrous or humid cultures, primary culture reproducing by arthrospores.

(*a*) Subgenus *Grubyella*. Type species: *A. schoenleini*.

(*b*) Subgenus *Bodinia*: smaller cultures which rapidly become pleomorphic. Type species: *T. violaceum*.

Outwardly the effect of this revision is to leave Grigoraki's system resembling that of Sabouraud. There is, however, a profound difference in regard to the characters on which the genera are based. Sabouraud relied on the spatial relations between parasite and host, while Grigoraki ignored these and emphasised characters on agar media.

Sabouraud (1929 *d*) summarised his matured views on these mycological classifications of the dermatophytes and presented a slightly revised version of his system. The mycological classifications are criticised as artificial, as they are based solely on morphology in culture. Dichotomous keys to species are likewise condemned in favour of a methodical and rational classification founded on the totality of characters. Sabouraud then enunciated several broad principles which guide systematic arrangement of these fungi. Toxins prepared from cultures of any *Achorion*, *Microsporon* or *Trichophyton* give identical skin reactions when tested on a patient sensitised by a previous attack of suppurating ringworm (kerion), while toxin from a *Sporotrichum* does not. This serological test shows that all the dermatophytes are closely related, and should not therefore be "scattered to the four winds of nomenclature." Different organisms regularly determine different maladies (thus an *Achorion* always produces favus) and this fact must have some value as a stable character in classification. Hairs taken from any one patient all show the same mode

of attack by the fungus when examined microscopically. A study of the parasitic morphology allows five distinct groups of dermatophytes to be distinguished. Sabouraud then examined each of the five groups into which he divided the dermatophytes in order to show the parallels between: (1) clinical characters, (2) parasitic structure, and (3) cultural characters. Sabouraud (1929 a) had already proposed to drop the names "trichophyton" and "ectothrix" from his classification in order to simplify the system. A "trichophyton" would then become merely a general term for a parasite invading the hair, as distinct from an "epidermophyton" which is confined to the stratum corneum. Sabouraud's five genera, *Endothrix*, *Microides*, *Microsporum*, *Achorion*, *Megaspore*, are analysed in the following scheme.

<div align="center">*Sabouraud's revised classification* (1929).</div>

Genus **Endothrix**.

Determines a ringworm with short broken hairs, chronic, usually healing spontaneously at puberty.

Parasitic structure: sporulating parallel hyphae, included within hair and filling it entirely.

Cultures (with the exception of *Endothrix* (*Trichophyton*) *violaceum*) powdery with aleuriospores only.

Genus **Microides**.

Almost always accompanied in man by an acute inflammatory reaction, and causing true kerion.

Parasitic structure: a sheath of sporulating filaments adhering to the hair, but retaining their filamentous arrangement.

Cultures: chalky in appearance; aleuriospores predominant; fuseaux sparse, short, extremity obtuse or truncated, comprising a small number of compartments, distaff-shaped, wall constricted at septa; pedicel large; fuseaux often bearing aleuriospores; spirals and nodular organs present.

Genus **Microsporum**.

Determines a ringworm with hairs 3–4 mm. long, enclosed in a white sheath; healing spontaneously at puberty.

Parasitic structure: a sheath of small spores distributed irregularly in a mosaic around hair.

Cultures: with racket hyphae; aleuriospores rare; fuseaux predominant, shuttle-shaped. In species of animal origin fuseaux with 7–15 compartments, never bearing aleuriospores, pedicel narrow, wall never constricted at septa. In human species fuseaux narrow, regular, rudimentary.

Genus **Achorion**.

Determines favus, characterised by scutula.

Parasitic structure: hair invaded by sinuous or rectilinear hyphae dividing by tri- or tetra-otomy.

Cultures: either glabrous, or in species of animal origin resembling the micro-sporums in the presence of numerous shuttle-shaped, pointed fuseaux.

Genus Megaspore[1].

Clinically may resemble either *Endothrix* or *Microides*.

Parasitic structure: a filamentous sheath, outside or invading hair, consisting of large spores $(5-7\mu)$.

Cultures: either downy with aleuriospores and a few rudimentary fuseaux, or glabrous and sterile.

Epidermophyton inguinale remains an isolated, but related species of dermatophyte.

A glabrous surface in cultures is regarded by Sabouraud as a parasitic degradation and not a sufficiently stable character on which to unite species in a genus. Sabouraud points out that three of the five groups based on clinical and microscopic characters are confirmed by mycological studies. These three genera, *Endothrix*, *Microsporum*, and *Microides*, can therefore be regarded as natural groups. The two remaining groups, *Achorion* and *Megaspore*, are less clearly established on cultural characters, as some members have glabrous cultures and only produce chlamydospores. Sabouraud concludes that it is better to conserve the dermatological system provisionally for these organisms, even if the morphological characteristics of two of the genera are not yet clear.

Langeron and Milochevitch (1930*b*) as a result of their studies of morphology on natural media, proposed a modification of the system of Ota and Langeron (1923). Their most revolutionary change was to unite four species: *Trichophyton asteroides* Sab., *T. granulosum* Sab., *T. radiolatum* Sab. and *T. interdigitale* Priestley and transfer them to a genus of the Gymnoascaceae as *Ctenomyces mentagrophytes* (Robin) Lang. and Mil. Their justification for this change lay in the "sterile perithecia" developed in their cultures on straw and grain. On these media powdery white or yellowish granules developed abundantly. Microscopically these globular bodies were seen to consist of downy masses bristling with spirals and covered with aleuriospores. In addition torulose hyphae similar to those described by Eidam for *Ctenomyces serratus* were present. The bodies exactly resembled the perithecia of a Ctenomyces, but lacked asci and ascospores. In spite of this deficiency Langeron and Milochevitch considered the resemblance sufficient to place all the dermatophytes definitely in the Gymnoascaceae. The following scheme is an outline of their system.

Langeron and Milochevitch's classification (1930).

Class ASCOMYCETES.

Order PLECTASCALES.

Family GYMNOASCACEAE.

Genus Ctenomyces Eidam. Microides characterised by sterile perithecia formed of crozier-like torulose hyphae terminating in spirals; by the conidial apparatus arranged in compound bunches; and by their distaff-shaped fuseaux. *C. mentagrophytes* (Robin) Lang. and Mil., *C. lacticolor* Sab.

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[1] Sabouraud's earlier term *Megalosporon* would appear preferable to the French "Megaspore."

Genus **Sabouraudites** (Ota and Lang.) Lang. and Mil. emend. Comprises all dermatophytes with the morphology of the old genus *Microsporon*, *i.e.* with spirals, numerous yellowish shuttle-shaped fuseaux, and a simpler conidial apparatus than *Ctenomyces*. *Sabouraudites felineus* (Fox and Blaxall), *S. gypseum* (Bodin), *S. quinckeanus* (Zopf), *S. gallinae* (Megnin), *S. audouini* (Gruby), *S. umbonatus* (Sab.).

Genus **Epidermophyton** (Lang.) Ota and Lang. emend. Conidial apparatus unknown, but possessing spirals and numerous fuseaux of a characteristic shape. A single species *E. floccosum* (Harz).

Genus **Trichophyton** (Malmsten) Lang. and Mil. emend. (nec. Ota and Lang.). Comprises all remaining dermatophytes. It is divided into sections corresponding to the genera recently established by Sabouraud:

(*a*) Endothrix.

(*b*) Megaspores.

(*c*) Epidermophytes corresponding to the former *Endodermophyton*, and Epidermophytons other than *E. floccosum*.

(*d*) Two species, which from lack of sufficient morphological data remain unclassified: *Achorion schoenleini* and *Microsporon ferrugineum*.

As a result of these modifications the generic names *Microsporon*, *Endodermophyton*, *Bodinia*, *Grubyella*, and *Ateleothylax* disappear from the nomenclature. Several criticisms may be levelled against this system. It is a system based solely on cultures, and the highly convenient and stable parasitic structure of these organisms is ignored. The result has been a somewhat academic system, though one of great interest. The genus *Sabouraudites* is simply the old genus *Microsporum*, and the latter name appears to be valid. The emended genus *Trichophyton* is admittedly an artificial group containing fungi with widely differing structure. Objection may also be made to the transfer of species to *Ctenomyces* when asci have still to be demonstrated.

Vuillemin (1931) elaborated his earlier classification. He retained his conception that there are, for the fungi, three permissible kinds of classification, arranged in descending order of significance: (1) a *normal classification*, the least arbitrary, based on organs of sexual reproduction; (2) an *auxiliary classification* based on asexual spores; and (3) a *medical classification*, based on the relation of the parasite with the tissues affected, a clinical expedient for rapid grouping. A summary of Vuillemin's system is given in the following scheme, in which the auxiliary genera to which he attributes various species are given in brackets.

Vuillemin's medical classification (1931).

Genus **Achorion**.

A. schoenleini, and *A. caninum* (Mycoderma), *A. quinckeanum*, *A. gypseum* and *A. gallinae* (Fusoma).

Genus **Microsporon**. Type species *M. audouini* Gruby.

(*a*) Section *Audouini*, parasites rendering the hair fragile near base of shaft: *Microsporon audouini*, *M. velveticum*, *M. tardum*, *M. umbonatum* (Mycoderma); *M. lanosum*, *M. audouini* var. *equinum*, *M. felineum* (Fusoma).

(*b*) Section *Mentagrophytes*, parasites which undermine the hair by dislocating the root, spores remaining in chains: *M. mentagrophytes*, *M. felis* (Mycoderma); *M. equinum* Vuillemin (Fusoma).

Genus **Trichophyton**. Parasites confined within the hair, the hair broken.

T. flavum, *T. tonsurans*, *T. currii*, *T. pterygoides* (Mycoderma).

Genus **Trichosporon**. A genus of parasites attacking hair in the tropics causing piedra.

T. giganteum Behrend 1890 (Mycoderma).

An inspection of the above classification shows that Vuillemin's genus *Trichophyton* corresponds to Sabouraud's *Endothrix*, while his *Microsporon* contains both Sabouraud's *Microsporon* and *Microides*. The classification has the advantage of conforming more closely to the rules of botanical nomenclature than other systems. Thus the genus *Trichophyton* is retained for a group correctly based on *T. tonsurans*, which is apparently an endothrix fungus, instead of renaming the group *Endothrix* as Sabouraud has done.

From the foregoing review of systems it will be apparent that the subject of classification is in a chaotic state, with little agreement on the limits of groups proposed or of the genus to which any particular species should be referred. It is possible to detect a conflict between workers who desire to conform to rules of botanical nomenclature and those who wish to retain terms that are familiar to medical workers. Again there is conflict between workers who base classification on microscopic morphology of cultures and those who admit the stable host-parasite relationship as a legitimate criterion for classification.

Of the various systems that have been proposed, those of Grigoraki have found relatively little acceptance. Ota and Langeron's now obsolete genera have been adopted by the National Conference on Nomenclature of Diseases (1933) for the United States in a report proposing a classification of diseases for hospital filing. In this system diseases are classified on a combined topographical and etiological basis. The suitability of a classification of the dermatophytes based on microscopic studies of cultures for filing clinical records may be questioned.

Sabouraud's original system was used without qualification by Bruhns and Alexander (1928) in their comprehensive survey of dermatomycoses, and it remains the most widely known of all the systems proposed.

The position is hard to summarise. The natural place for the dermatophytes seems to be among the Fungi Imperfecti. Although a very good case has been made out for their affinities with the Gymnoascaceae, until the ascogenous stage is regularly available it seems premature to speak of *Gymnoascus gypseus* or of *Ctenomyces mentagrophytes*. Classification has the double function of providing an index system to a difficult and important group of organisms, and at the same time of approaching as nearly as possible to a phylogenetic system paying due regard to priority. Vuillemin gave up the attempt and recognised distinct medical and botanical classifications. Sabouraud, however, has shown that the construction of a system satisfying both requirements is not so far distant as it once appeared to be, and three of his five

genera appear to be natural groups. His genus *Achorion*, uniting as it does *A. schoenleini* with *A. gypseum*, a fungus which is a typical *Microsporum* when in culture, certainly appears unsatisfactory to the botanist. The genus *Endothrix* may be invalid, as *Trichophyton* Malmsten appears to be an older name for an *Endothrix* fungus. On the other hand Sabouraud's revised system is an achievement in simplification and co-ordination that deserves to be carefully considered before it is rejected in favour of an unfamiliar system based on purely cultural characters. Slight adjustments would serve to bring Sabouraud's system into accordance with the rules of botanical nomenclature.

VI. SUMMARY.

The dermatophytes are a group of fungi which parasitise animals by invading the keratinised portions of the epidermis. They appear to be unable to attack internal organs and are apparently dermatotropic. Ringworm in its various forms is a primary localised dermatophyte infection.

The concept of the activity of the dermatophytes has been enlarged by the recognition that they can also determine secondary non-parasitic skin lesions on distant parts of the body. Several records of isolations of these fungi from the blood indicate that spores may be carried from the primary lesion to other parts of the body by means of the blood stream.

Hairs infected by certain species of dermatophytes show a green fluorescence in ultra-violet light not possessed by uninfected hairs. This phenomenon can be utilised to detect infected hairs among normal ones. The fluorescence is due to a water-soluble substance which can be extracted from infected hairs.

The wealth of spores and other organs produced in cultures on certain animal and vegetable media suggests that these fungi have a natural saprophytic stage in their life history. It is also possible that the saprophytic stage occurs on naturally infected hairs after they fall from the animal body.

The dermatophytes in culture are subject to variation. The particular irreversible degenerative mutation known as "pleomorphism" can take place in isolates derived from either a single aleuriospore or a single fuseau. Pleomorphism appears to be due to a toxic action on the organism of monosaccharides and disaccharides. In media containing polysaccharides pleomorphism is not observed.

Asci have been reported by several workers. While certain of these records appear to be unsubstantiated, others point to an affinity between the dermatophytes and the Gymnoascaceae. The classification of the 200 or more species is in a state of chaos. The newer systems show few advantages over Sabouraud's revised classification.

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DIE KOORDINATION DER BEWEGUNG BEI DEN ARTHROPODEN IN ABHÄNGIGKEIT VON ZENTRALEN UND PERIPHEREN BEDINGUNGEN

Von ERICH VON HOLST.

INHALT.

I. PROBLEM UND METHODE, ANGEWANDTE BEZEICHNUNGEN.

DAS Teilgebiet aus der Physiologie des Zentralnervensystems, über das dieser Aufsatz berichtet, wird noch nicht lange bearbeitet. Gleichwohl verdienen die hier zusammengefassten Erscheinungen allgemeine Beachtung, und die an sie geknüpften Gedankengänge beginnen bereits einen merklichen Einfluss auf die Vorstellungen zu nehmen, die man sich über die schwierigen und verwickelten Vorgänge im Zentralnervensystem bildet. Der Grund dafür ist nicht in der *Methode* zu suchen, die hier angewendet wird; sie ist meist einfach, enthält nichts grundsätzlich Neues, und alle Versuche sind leicht genug zu wiederholen. Das hat den Vorteil, dass über die Erscheinungen selbst kaum Meinungsverschiedenheiten bestehen können—das Problem liegt einzig und allein in der *Deutung* der Versuche; und hier sind in der Tat z. T. ganz neuartige Vorstellungen entstanden, Vorstellungen, die insbesondere durch die Gedankenarbeit Bethes sich zu einer umfassenden Lehre verdichtet haben, der Lehre von der *Plastizität des Nervensystems* (Bethe, 1925, 1931 *a*, 1933).

Der vorliegende Aufsatz beschränkt sich auf die Arthropoden, weil einmal hier die experimentelle Arbeit am weitesten fortgeschritten ist und weil andererseits bei

dieser Gruppe die vollständigsten und zugleich einfachsten gedanklichen Voraus-
setzungen für die theoretische Seite des Problems liegen.

Die Beschäftigung mit dieser Tiergruppe macht eine genauere Beschreibung
der Haltungs- und Bewegungskoordination, insbesondere der verschiedenartigen
gegenseitigen Beziehung der Beinbewegungen notwendig. Die Bewegung des
laufenden Beines lässt sich in zwei Phasen zerlegen: die "Stemmphase," in der es
dem Boden aufgestemmt und nach hinten bewegt wird, und die "Schwingphase,"
in der es vom Boden erhoben und wieder nach vorne gebracht wird. Beide Phasen
wechseln miteinander ohne Zwischenpause ab. Eine Stemm- und eine Schwing-
phase bilden zusammen einen Schritt. Wenn zwei Beine beim Laufen immer genau
übereinstimmend, synchron, bewegt werden, ist ihr "Phasenabstand" = o; nehmen
sie immer die entgegengesetzte Lage ein, alternieren sie wie das rechte und linke
oder die beiden gleichseitigen Beine eines trabenden Pferdes, so ist ihr Phasenab-
stand = 1/2 Schritt. Zwischen diesen Extremen gibt es alle Übergänge. Wenn z. B.
bei einer Anzahl hintereinander liegender Beine jedes mit seinem Nachbarn einen
Phasenabstand von 1/6 Schritt innehält, läuft das erste mit dem vierten alternierend,
mit dem siebenten synchron; die Beine nehmen insgesamt eine wellenförmige
Anordnung ein, und diese "Wellen" verschieben sich beim Laufen über die Beine
nach hinten oder nach vorne, je nachdem, ob das jeweils weiter hinten gelegene
Bein dem davorliegenden in der Bewegung um 1/6 Schritt voraus oder hinter ihm
zurück ist.

II. DIE NORMALE KOORDINATION DER BEINBEWEGUNG UND IHRE VERÄNDERUNG DURCH AMPUTATION VON BEINEN BEI LAUFENDEN FORMEN.

Alle Arthropoden, die man bis jetzt daraufhin untersucht hat, bewegen ihre
Extremitäten beim Laufen in einer ganz bestimmten Anordnung. Ob es überhaupt
Formen gibt, bei denen jedes Bein vom anderen ganz unabhängig läuft, ist nicht
bekannt und es ist auch nicht wahrscheinlich.

Dass diese *Ordnung nicht starr* festliegt, sondern von der Tätigkeit der laufenden
Beine selbst abhängig ist, und dass sie *nach Verlust von Beinen gesetzmässig* einer
neuen Ordnung Platz machen kann, haben zuerst v. Uexküll (1909) und v. Budden-
brock, der eine an Krebsen (Crustaceen), der andere an Insekten beschrieben. Am
einfachsten liegen die Dinge bei den Insekten.

Insekten. Die überwiegende Mehrzahl aller Insekten läuft in der Weise, dass
je drei Beine miteinander synchron und mit den anderen drei Beinen alternierend
bewegt werden; und zwar laufen das erste und dritte Bein einer Seite mit dem
zweiten der gegenüberliegenden Seite gemeinsam (Abb. 1, I *a*). V. Buddenbrock
(1921) fand nun, an der Stabheuschrecke *Dixippus*, dass Entfernen einer Extremität
an dieser Ordnung nichts ändert, dass aber nach Verlust von zwei Beinen sofort
eine Neuordnung eintreten kann, wie sie Abb. 1, I *b–f* schematisch veranschaulicht.
Die übrigbleibenden vier Beine werden so gesetzt, wie die eines trabenden Pferdes
—es stellt sich immer *Kreuzgang* ein. Im Falle von Abb. 1, I *b* entspricht das auch

der ursprünglichen Ordnung. Bei *c* und *d* weicht es von ihr z. T. ab, bei *e* und *f* aber ist die neue Ordnung der alten völlig entgegengesetzt: Hier laufen das erste und das dritte Bein einer Seite, die sonst synchron arbeiten, alternierend (*e*) und zwei sonst alternierend arbeitende Beinpaare synchron (*f*).

Übereinstimmende Beobachtungen wurden später auch an anderen Insekten (Coleopteren, Bethe und Woitas, 1930 *b*) gemacht.
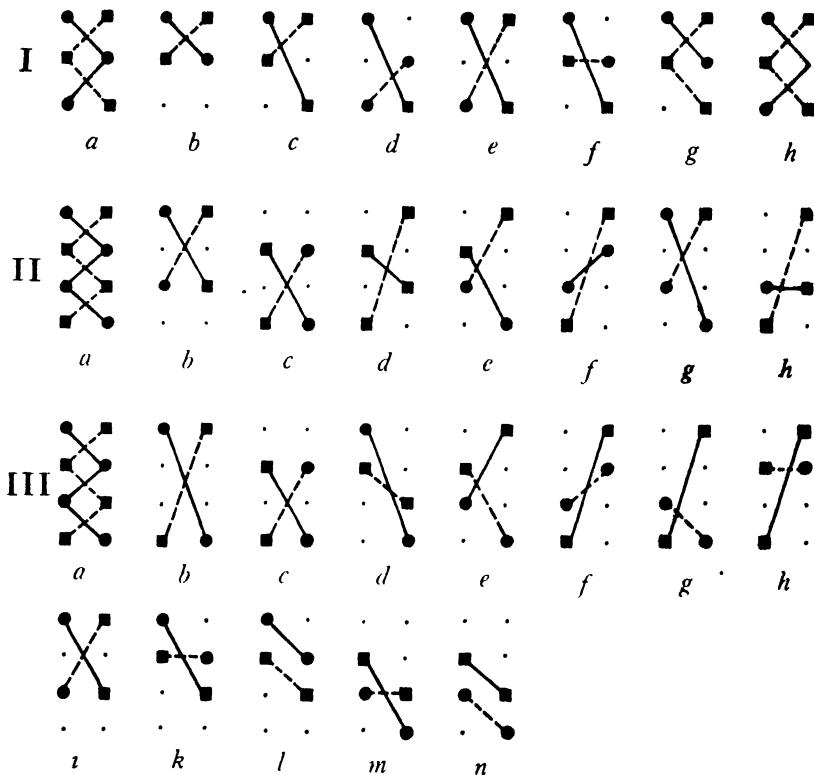
Abb. 1. Schematische Darstellung der Beinkoordination beim Laufen, vor und nach Amputation von Beinen, an verschiedenen Arthropoden. Die beim intakten Tier synchron bewegten Beine sind in allen Schemata mit übereinstimmenden Zeichen (Kreisen bzw. Quadraten) versehen. Die in jedem Einzelfall synchron laufenden Beine sind durch einfache bzw. unterbrochene Striche verbunden; die kleinen Punkte bedeuten amputierte Beine.
     I. *Dixippus* (nach v. Buddenbrock); II. *Opilio*; III. *Carcinus* (nach Bethe, etwas verändert, II *a* und III *a* vereinfacht).

Arachnoiden. Unter den Spinnen mit ihren vier Beinpaaren sind die *Opilioniden* (Bethe, 1930 *a*) näher untersucht worden. Ihre normale Koordination stimmt im wesentlichen mit der der Insekten überein: das erste und dritte Bein der einen wird mit zweiten und vierten der anderen Seite synchron, mit den übrigen vier Beinen alternierend bewegt (Abb. 1, II *a*). Die Synchronie der zusammenarbeitenden vier Beine ist allerdings nicht vollkommen genau; das vorderste Bein geht in der

Bewegung um den Bruchteil einer Schrittphase voraus, das letzte bleibt ein klein wenig zurück.

Die wichtigsten Koordinationsumstellungen nach Beinamputation zeigt Abb. 1, II *b–h*. Die Hälfte der Beine ist entfernt, sodass immer vier Beine übrigbleiben: der Gang ist auch hier in allen Fällen ein Kreuzgang. Bei *f* entspricht das der alten Ordnung, in den übrigen Fällen ist eine mehr oder weniger grosse Umstellung eingetreten. Am weitesten geht diese bei *b*, *c* und *e*, wo je zwei ursprünglich synchron bewegte Beine jetzt miteinander alternieren. Alle diese Umstellungen erfolgen, ebenso wie bei *Dixippus*, augenblicklich nach der Operation.

Crustaceen. Bei den Krebsen wissen wir über den Gang der *Decapoden* genauer Bescheid; am eingehendsten sind hier die Krabben (Brachyuren) durch Bethe (1930 *a*) studiert worden. Die normale Tätigkeit der vier Beinpaare eines *Carcinus* ist in gewissen engen Grenzen variabel, sie geht aber auf das gleiche Grundschema zurück, das auch die Opilionen zeigen. Die Beine jedes Segmentes alternieren, und ebenso alterniert das erste Bein einer Seite mit dem zweiten, das zweite mit dem dritten, und dieses mit dem vierten Bein derselben Seite. Die Komplikation besteht darin, dass der Phasenabstand von einem zum nächsten Bein nicht immer genau 1/2 Schritt beträgt, sondern sich etwas (bis auf 1/3 Schritt) verringern kann. Diese Koordination gilt sowohl für das Vorwärts- als auch für das viel häufiger ausgeübte Seitwärtslaufen nach links oder rechts.

Die Amputation von Beinen bringt Ergebnisse, die mit den bisherigen aufs beste übereinstimmen. Am interessantesten sind die Kombinationen, bei denen im ganzen vier Beine erhalten sind: man ersieht aus Abb. 1, III *b–i*, dass die neue Koordination auch hier wieder der Kreuzgang ist, wobei die ursprüngliche gegenseitige Ordnung der Beine weitgehend belanglos ist. *k–n* sind Kombinationen, die bis zu einem gewissen Grade als Ausnahmen dieser Regel gelten können, insofern als hier die Koordination von *k* mit der von *l* und die von *m* mit der von *n* abwechseln kann.

Für die *Makruren* gilt, soweit die Koordination der Beine, die hier schwieriger im Film festzuhalten ist, sichergestellt werden konnte, im grossen und ganzen das gleiche wie für die Brachyuren (Bethe, 1930 *a*)—sowohl hinsichtlich des normalen Ganges als auch der Umordnung der Beine nach ihrer teilweisen Amputation.

Wenn, man aus den bisherigen Versuchen das allgemein Gültige zusammenfasst, so würde es lauten, dass erstens *aufeinanderfolgende Beine einer Seite alternieren*, auch wenn sie am intakten Tier synchron laufen würden, und dass zweitens *über Kreuz gelegene Beine synchron arbeiten*. Dabei scheint die räumliche Entfernung, die Frage, ob zwei miteinander alternierende Beine aufeinander unmittelbar folgen, oder ob beinlose Segmente dazwischen liegen, eigenartigerweise belanglos zu sein.

Wenn die Arthropoden mit drei oder vier laufenden Beinpaaren trotz entfernter systematischer Verwandtschaft eine weitgehende Ähnlichkeit in ihrem Koordinationsverhalten unter den besprochenen experimentellen Bedingungen zeigen, so ist die Frage interessant, wie sich unter den gleichen Bedingungen die vielfüssigen Arthropoden verhalten werden. Unter ihnen ist zuerst der Diplopode *Iulus* (Bethe und Mitarbeiter, 1931 *c*, 1933) eingehend untersucht worden; er und ebenso gewisse

Chilopoden (v. Holst, 1934 *c*) zeigen aber ein grundsätzlich abweichendes Koordinationsprinzip, sodass sich ihre Besprechung an anderer Stelle empfiehlt.

Dagegen fügt sich der Chilopode (Hundertfüsser) *Lithobius* den Holst (1934 *a*) untersucht hat, gut in die bisherigen Formen ein, und sein Studium bringt mancherlei weiteren Aufschluss. Im normalen Lauf, den Abb. 3 *a* zeigt, setzt
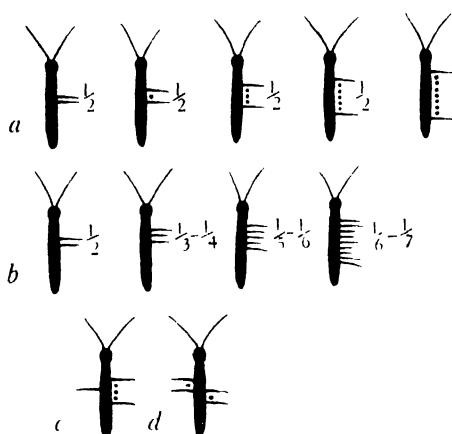
Abb. 2.
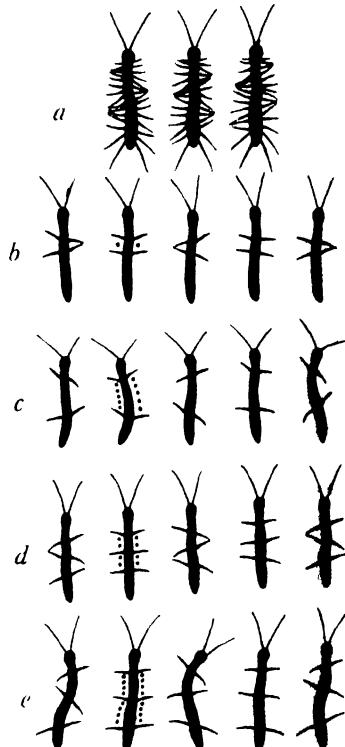
Abb. 3.

Abb. 2. Schemata der Beinkoordination bei *Lithobius*; die vorhandenen Beine sind eingezeichnet, die zwischen ihnen gelegenen beinlosen Segmente durch Punkte angedeutet. Die Phasenabstände von einem zum nächsten Bein sind in Reihe *a* und *b* daneben geschrieben (z. B. 1/2 Schritt, 1/6 Schritt). Bezüglich *c* und *d* siehe Text. (Nach Holst.)

Abb. 3. Laufende *Lithobii* mit verschiedenen Beinkombinationen (nach Filmen von Holst): *a*, intaktes Tier; *b* und *c*, zwei Tiere mit je zwei Beinpaaren; *d* und *e*, zwei Tiere mit je drei Beinpaaren. Im zweiten Bild jeder Reihe ist die Zahl der zwischen den Beinen gelegenen beinlosen Segmente durch Punkte angedeutet; die Reihen sind von links nach rechts zu lesen.

Lithobius die zwei Beine jedes seiner fünfzehn Beinpaare, von denen allerdings das kürzeste erste und die zwei langen letzten Paare sich selten am Laufen beteiligen, alternierend auf. Die aufeinanderfolgenden Beine einer Seite haben einen ganz dichten Phasenabstand, der zwischen 1/6 und 1/7 Schritt schwankt; und zwar ist immer das jeweils weiter hinten gelegene Bein dem davorgelegenen um 1/6 bis 1/7 Schritt voraus, sodass eine wellenförmige Anordnung entsteht. Die "Wellen"

laufen von hinten nach vorn über den Körper, weil die Lage, die das letzte laufende Bein jeweils innehat, unmittelbar darauf vom vorletzten, dann vom drittletzten usw. eingenommen wird, sodass der Blick fortwährend von hinten nach vorn gelenkt wird. Der Körper selbst vollführt bei schnellerem Lauf eine mit den Beinen koordinierte Wellenbewegung.

Die Koordinationsumstellungen, die auch hier augenblicklich auftreten, sind am übersichtlichsten, wenn mit der kleinsten Zahl übriggelassener Beine begonnen wird. Man kommt auf diesem Wege zu einigen einfachen Regeln, in die sich alle Kombinationen einfügen lassen. Wenn alle Beine bis auf zwei, die auf der gleichen Seite liegen, entfernt sind, so alternieren diese beiden miteinander (Abb. 2 *a*) und zwar sowohl wenn sie einander benachbart, als auch wenn ein bis fünf Segmente dazwischen gelegen sind. Ist der Zwischenraum noch grösser, dann geht die Koordination überhaupt verloren, beide Beine laufen nun in einem voneinander unabhängigen Takt, wobei der Rhythmus des vorderen gegenüber dem hinteren Bein beschleunigt zu sein pflegt. Auch bei drei einseitig erhaltenen Beinen alterniert noch jedes mit dem anderen; eine Ausnahme bildet hier der Fall, wenn sie unmittelbar aufeinander folgen: der Phasenabstand wird hier geringer, statt 1/2 etwa 1/3 bis 1/4 Schritt.

Sind mehr als drei Beine einseitig erhalten, so verändern sich die Koordinationsbeziehungen umso mehr, je mehr Beine erhalten bleiben. Abb. 2 *b* zeigt, dass die Phasenabstände bei unmittelbarer Aufeinanderfolge der Beine für zwei Beine = 1/2 Schritt, für deren drei = 1/3 bis 1/4, für fünf Beine 1/5 bis 1/6, für neun und mehr Beine 1/6 bis 1/7 Schritt betragen. Das ganz entsprechende gilt auch, wenn zwischen den Beinen Lücken von z. B. je einem Segment vorhanden sind, nur dass hier die Verringerung des Phasenabstandes kein so kleines Mass erreicht.

Die Koordination ist die gleiche, wenn die zugehörigen gegenüberliegenden Beine erhalten bleiben. Das bedeutet, dass bei zwei Beinpaaren mit einem Zwischenraum bis zu fünf Segmenten Kreuzgang auftritt, wovon Abb. 3 *b* und *c* Beispiele nach Filmen gibt; und dass bei drei Beinpaaren mit Zwischenräumen von je ein bis vier Segmenten sich der typische Insektengang einstellt, wie Abb. 3 *d* und *e* ihn zeigt. Sind vier Beinpaare erhalten, so stimmt die Koordination bei Zwischenräumen von je ein, zwei oder drei Segmenten annähernd mit derjenigen der untersuchten Crustaceen und Arachnoiden überein: der Phasenabstand beträgt 1/2 bis 1/3 Schritt (bei *Carcinus* 1/2 bis 1/3, bei *Opilio* 1/2 Schritt oder etwas weniger).

Es ergibt sich also die bemerkenswerte Tatsache, dass man durch Reduktion der Beinzahl bei *Lithobius* aus der so andersartigen wellenförmigen Beinordnung zu einer Koordinationsform kommt, die immer mit der Koordination derjenigen Arthropoden annähernd übereinstimmt, die ebenfalls diese Zahl von Beinen besitzen. Das anatomische Lageverhältnis, die Entfernung der Beine voneinander ist dabei zwar nicht völlig, aber doch in weiten Grenzen belanglos.

Darüber hinaus lassen sich aber an *Lithobius* auch die Koordinationsumstellungen, die wir für die anderen Arthropoden besprochen haben, realisieren. So kann man z. B. an einem zum "Insekt" gemachten *Lithobius* (etwa Abb. 3 *d*) die Umstellungen, die Buddenbrock an *Dixippus* durch Beinamputation erzielte, mit

demselben Erfolge (von einer belanglosen Abweichung abgesehen) auch hervor-
rufen.

Die Befunde an *Lithobius* lassen sich in *zwei Regeln* etwa folgenden Inhalts
fassen:

*Sind insgesamt wenige—nicht mehr als drei—Beine bezw. Beinpaare hintereinander
gelegen, so besteht zwischen ihnen der weitest mögliche Phasenabstand (1/2 Schritt).*

*Sind mehr als drei Beine oder Beinpaare vorhanden, so verringern sich die Phasen-
abstände umso mehr, je mehr Beine insgesamt hintereinander gereiht liegen.*

Noch eine weitere Beobachtung ist (nach Holst) für das Verständnis des
Koordinationsmechanismus von Bedeutung: die Ordnung der Beine wird bei
Lithobius und auch bei den übrigen Formen niemals mechanisch streng festge-
halten. Weder die Ordnung des intakten noch die des z. B. vier- oder sechsbeinigen
Lithobius ist unverrückbar festgelegt, sondern sie stellt nur das "Gleichgewicht"
dar, dem die Beine immer zustreben, wenn sie zu laufen beginnen oder durch
mechanische Ursachen in ihrer Laufordnung gestört sind. Es besteht gleichsam
ein "Gefälle," das seinen Ausgleich erst bei der für die betreffende Beinkombination
geltenden Ordnung findet. Dieses Gefälle ist nun verschieden stark und zwar
immer am schwächsten in den Grenzgebieten einer für diese Beinzahl geltenden
Ordnung. So kommt z. B. der vierbeinige *Lithobius* am leichtesten bei einem
Zwischenraum von fünf Segmenten aus diesem Ordnungsgleichgewicht; der sechs-
beinige findet seine Ordnung (nach einer Störung oder beim Laufbeginn) am
langsamsten bei einem Zwischenraum von je vier Segmenten.

Es gibt sogar Kombinationen, bei denen eine endgültige Ordnung sich überhaupt
nicht einstellt (s. auch für *Carcinus* Abb. 1, III *k–n*). Zwei Beispiele zeigt Abb. 2 *c, d*;
in *c* alterniert das erste mit dem zweiten rechten Bein, während das linke bald mit
dem ersten, bald mit dem zweiten der rechten Seite alterniert. In *d* laufen das erste
linke und das zweite rechte Bein synchron; das zweite linke und das erste rechte
laufen bald alternierend in der herkömmlichen Weise, bald auch synchron, wie es
im Sinne des Kreuzganges zu erwarten ist. In solchen Fällen liegen, so kann man
sagen, zwei einander entgegengesetzte Ordnungstendenzen miteinander in Konflikt
—der Ordnungszustand bleibt dann labil.

III. DIE KOORDINATION SCHWIMMENDER FORMEN.

Der Mechanismus der Beinordnung bei den bisher besprochenen laufenden
Arten ist, wie es nach allem scheint, auf gleiche oder ähnliche Grundsätze aufgebaut.
Bei Arten, die eine andere Fortbewegungsweise besitzen, werden diese Prinzipien
bis zu einem gewissen Grade geändert sein müssen. Das ist z. B. zu erwarten von
springenden Arthropoden, wie den Locustiden, oder von grabenden, wie manchen
Grylliden oder Coleopterenlarven und auch von den schwimmenden Arten. Unter
diesen letzteren sind die Verhältnisse für einige Käfer (Coleopteren) und Krabben
(Brachyuren) bekannt geworden.

Coleopteren. Von den beiden von Bethe und Woitas (1930 *b*) untersuchten
Schwimmkäfern *Hydrophilus* und *Dytiscus* bewegt der eine, *Hydrophilus piceus*,

seine Beine beim Schwimmen noch in sehr ähnlicher Weise wie die laufenden Insekten. Er benutzt das zweite und dritte Beinpaar und bewegt diese vier Beine im Kreuzgang, jedes mit dem anderen alternierend. Nach Verlust eines Beines bleibt die Koordination noch unverändert, nur macht das auf der betreffenden Seite übrigbleibende Bein entsprechend stärkere Bewegungen, sodass die gradlinige Fortbewegung gewahrt bleibt. Auch nach Verlust eines Beinpaares arbeitet das übrigbleibende in der alten Weise weiter. Sind aber beide Hinter- und das rechte Mittelbein amputiert, dann beginnt bald das vorher unbewegliche rechte Vorderbein sich am Rudern zu beteiligen, wobei es aber im schnelleren Rhythmus schlägt, als das erhalten gebliebene linke Mittelbein. Nach Entfernung aller vier Schwimmbeine treten die beiden Vorderbeine, indem sie synchron oder alternierend schlagen, in den Dienst der Fortbewegung.
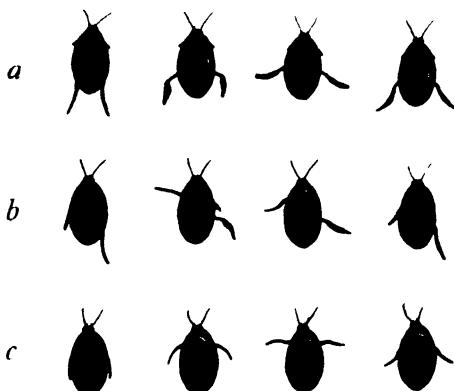
Abb. 4. Schwimmender *Dytiscus* (nach Filmen von Bethe): *a*, normales Tier; *b*, nach Amputation des linken Hinterbeines; *c*, nach Entfernung beider Hinterbeine.

Interessanter als diese Umstellungen bei *Hydrophilus* sind diejenigen, die man bei *Dytiscus marginalis* erhält. Die normale Schwimmweise zeigt Abb. 4 *a*: es rudert nur das letzte Beinpaar, und zwar synchron, die übrigen Beine liegen dem Körper an. Wird eines dieser zwei Schwimmbeine amputiert, so tritt sofort das gleichseitige Mittelbein in Aktion und arbeitet mit dem gegenüberliegenden Schwimmbein zusammen, wie Abb. 4 *b* zeigt. Entfernt man beide Schwimmbeine, so übernehmen unmittelbar darauf beide Mittelbeine die Schwimmtätigkeit, indem sie gerade so rudern, wie vorher die Schwimmbeine (Abb. 4 *c*). Nach Verlust der zwei hinteren Beinpaare treten sogar die kleinen Vorderbeine in Tätigkeit, indem sie synchron oder alternierend rudernd den Körper langsam durch das Wasser bewegen.

Crustaceen. Bei den Crustaceen sind wir über die Schwimmtätigkeit der Portuninen oder Schwimmkrabben *Portunus* und *Polybius* durch Kühl (1933) unterrichtet. Diese Tiere zeichnen sich schon anatomisch dadurch aus, dass das letzte Beinpaar in charakteristischer Weise zum Schwimmen umgebildet ist: die Glieder sind verkürzt, plattenförmig verbreitert, und ein Drehgelenk ermöglicht

kreiselnde Schwingung des Beines. Auf dem Boden laufen die Tiere in der für
Insekten üblichen Weise auf den drei vorderen Beinpaaren; die Schwimmbeine
werden beim Laufen auch nach Verlust aller Schreitbeine nicht verwendet. Bei
schnellem Lauf über den Boden beginnen sie synchron zu schwingen, aber in
einem anderen viel schnelleren Rhythmus. Beim Schwimmen werden die Schreit-
beine meist gestreckt gehalten, bei *Polybius* aber auch, und zwar wiederum in
einem anderen Rhythmus als die Schwimmbeine, mitbewegt. Nach Verlust aller
Gangbeine ist das Schwimmen kaum beeinträchtigt. Nach Verlust beider Schwimm-
beine dagegen sind die Tiere ausserstande zu schwimmen. Nach Amputation nur
eines Schwimmbeines ist der Erfolg zunächst eine Fortbewegung im Kreise, aber
bald wird durch Veränderung der Beinstellung wieder gradliniges Schwimmen
ermöglicht.

Unter den besprochenen schwimmenden Formen ist die Koordination von
Hydrophilus durchaus am ursprünglichsten; sie stimmt fast noch völlig mit dem
Insektengang überein. Bedeutend abgewandelt ist die Beinordnung von *Dytiscus*.
Hier treten zwei neuartige Erscheinungen auf: die Synchronie der Beine eines
Segments und die Ersetzbarkeit eines Beines durch andere, die beim intakten Tiere
überhaupt nicht an dieser Bewegung teilnehmen. Am weitesten spezialisiert ist die
Schwimmbewegung der Krabben. Hier findet sich überhaupt keine Form einer
Bewegungskoordination zwischen Gang- und Schwimmbeinen mehr; und so fehlt
auch das vikariierende Eintreten der einen Beinart für die andere.

IV. HALTUNGSÄNDERUNG DER RESTLICHEN BEINE NACH BEINVERLUST.

Mit der Änderung der Bewegungskoordination nach Beinamputation geht
häufig auch eine Veränderung der Haltung Hand in Hand, eine Umstellung des
gegenseitigen Lageverhältnisses der übrigbleibenden Beine. Diese Änderung der
Beinstellung wird immer dann auffällig, wenn die amputierten Gliedmassen für die
Aufrechterhaltung des Gleichgewichtes von Bedeutung waren.

Bei *Lithobius*, dessen abgeflachter Körper auch ohne Beine keine Neigung zum
Umfallen zeigt, werden solche Stellungsänderungen daher gänzlich vermisst.

Bei *Carcinus* sind sie schon bei vielen Beinkombinationen deutlich. Am auffäl-
ligsten sind aber die Stellungsänderungen bei *Opilio*, der zur Aufrechterhaltung der
Normallage auf die Unterstützung durch die langen Beine angewiesen ist. Abb. 5
zeigt die normale Haltung und die Stellung der Beine bei einigen Kombinationen.
Sie werden immer nach Möglichkeit in die Richtung gewendet, wo durch Ausfall
eines Beines ein Unterstützungspunkt verloren gegangen ist.

Auch bei den *schwimmenden Formen* stellt sich eine veränderte Haltung und
Bewegungsrichtung der Beine immer dann ein, wenn die Aufrechterhaltung der
symmetrischen Lage und der gradlinigen Fortbewegung es erfordern. Sowohl die
Schwimmkrabben (Portuninen) als auch der Schwimmkäfer *Dytiscus* können sich
nach Verlust eines Schwimmbeines noch richtig fortbewegen. Das übrigbleibende
Bein schwingt dann in einer veränderten Ebene und an der Steuerung beteiligen

sich auch die vorderen nicht aktiv bewegten Beinpaare, indem sie eine unsymmetrische Haltung einnehmen.

In diesen Abschnitt gehört schliesslich auch eine alte Beobachtung Bethes (1897 *a*), die die Art und Weise betrifft, wie der auf dem Rücken liegende *Carcinus* wieder zur Bauchlage zurückkehrt. Hierzu verwendet er das letzte Beinpaar, das weit unter den Rücken geschoben wird und den Körper über das Abdomen zurückdreht. Dieser sogenannte "*Lagereflex*" wird nun nach Amputation des letzten vom vorletzten Beinpaar, nach dessen Entfernung vom drittletzten in derselben Weise ausgeführt. Nach Entfernung aller übrigen Beine bringt sogar das erste Beinpaar ihn zustande. Auch wenn die Beine unsymmetrisch entfernt sind, wird der Lagereflex sofort ausgeführt, und zwar arbeiten genau wie bei symmetrischer Amputation immer die beiden auf jeder Seite am weitesten nach hinten gelegenen Beine zusammen.
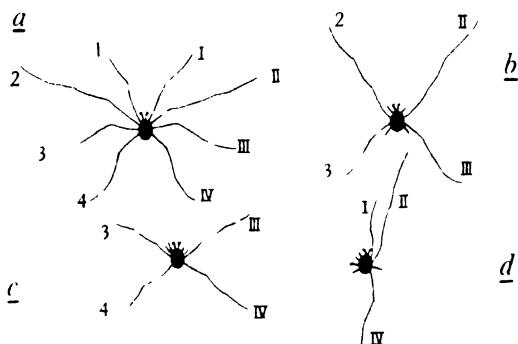
Abb. 5. Haltung der Beine bei *Opilio*: *a*, normales Tier; *b*, nach Entfernen des ersten und vierten; *c*, nach Entfernen des ersten und zweiten Beinpaares; *d*, nach Amputation des dritten rechten und aller linken Beine. (Die Beine sind links mit arabischen, rechts mit römischen Zahlen bezeichnet.) (Nach Bethe.)

Dieses Verhalten erinnert an das Schwimmen von *Dytiscus*: hier wie dort sind es immer die zwei jeweils letzten Extremitäten, die sich zu einer Arbeitseinheit zusammenfinden.

V. ANALYSE DER EINZELNEN AN DER KOORDINATION BETEILIGTEN FAKTOREN.

Die Beschreibung der Koordinationsumstellungen bei Verlust von Beinen hat zur Aufstellung einiger einfacher *Regeln* geführt, die eine gewisse Allgemeingültigkeit beanspruchen. Eine solche Auffassung ist indessen bis jetzt noch nicht gerechtfertigt; denn wir kennen weder die verschiedenen äusseren und inneren Faktoren, die für die normale Koordination eine Bedeutung haben können, noch ist bekannt, in welcher Weise diese durch Beinamputation verändert werden, oder welche neuartigen Bedingungen ein derartiger Eingriff schafft.

Man könnte z. B. einwenden, dass die mechanischen Bedingungen für die Bewegung der übrigbleibenden Gliedmassen vielleicht so verändert werden, dass sie eine neue Ordnung der Beine von selbst nach sich ziehen, ohne dass eine zentralnervöse Umstellung stattgefunden hätte. In diesem Falle würden die gewonnenen Koordinationsregeln keine physiologischen Eigenschaften des Nervensystems, sondern in erster Linie anatomische Eigenheiten des Körpers und der Gliedmassen betreffen. Dieser und ähnliche Einwände sind durchaus berechtigt, und darum alle Faktoren, die bei der Bewegungskoordination eine Rolle spielen können, zu prüfen.

Von der grössten Wichtigkeit für das Zustandekommen einer Laufbewegung überhaupt ist die Berührung der Extremitäten mit dem *Boden*, worauf Kühl (1933) als erster hinwies. Das gilt für alle Arthropodengruppen.

Wenn ein laufendes Bein in der Stemmphase keinen Boden findet, so scheidet es sofort aus der Ordnung der übrigen aus und beginnt gewöhnlich, nach unten hin rhythmisch auszugreifen, tritt in eine andere Bewegungsform, die "Greif"- oder "*Such*"-bewegung (Holst) ein. Ist vielen oder allen Beinen der Boden entzogen, so führen alle diese Suchbewegung aus, wobei eine gegenseitige Koordination vermisst wird. Bei *Lithobius* z. B. bewegt sich dann jedes Bein in einem von den anderen, auch dem eigenen Partner auf der anderen Körperseite, unabhängigen Rhythmus.

Die nähere Untersuchung dieser Suchbewegung an *Lithobius* und anderen Chilopoden (Holst, 1934 *c*) hat zur Auffassung geführt, dass sie auf einen sogenannten "automatischen" Prozess im Nervensystem zurückgeht, d. h. dass zu ihrer Auslösung nicht Erregungszufuhr aus der Peripherie erforderlich ist; und dass ferner jedes einzelne Ganglion der Bauchkette über diese automatische Fähigkeit verfügt; denn die zwei Beine irgendeines isolierten einzelnen Körpersegments führen diese Bewegung ebenfalls aus, sobald das Segment vom Boden erhoben wird. Der wirksame Reiz, der bei Berührung des Beines mit dem Boden die Suchbewegung hemmt, bezw. in Schreitbewegung umwandelt, ist aber nicht der Kontaktreiz selbst; denn wird die Beinspitze mit einer kleinen Wachskuppe umgeben, so führt das Bein bei Entziehung des Bodens trotz Bestehenbleiben seines Kontakts mit der Umgebung sofort Suchbewegung aus, und umgekehrt unterbleibt diese bei Hochheben des Beines vom Boden mittels eines vorher seitlich befestigten Haares.

Der entscheidende Faktor ist also das Vorhandensein oder Fehlen einer gewissen Belastung, und die fraglichen Rezeptoren sind *Propriozeptoren*, die wahrscheinlich in den Beinmuskeln sitzen und durch deren Anspannung gereizt werden. Die Erregung dieser Muskelrezeptoren ist demnach eine unentbehrliche Vorausbedingung für die Laufbewegung des einzelnen Beines.

Bei den schwimmenden Formen mit abweichender Beinkoordination wie den Schwimmkrabben und dem Schwimmkäfer *Dytiscus* scheint die Suchbewegung zu fehlen, an ihre Stelle tritt die Schwimmbewegung. Bei *Hydrophilus* (Bethe, 1897 *b*) und bei den Portuninen z. B. löst der fehlende Kontakt mit dem Boden sofort Schwimmen aus (Kühl).

Es ist durchaus denkbar, dass die Schwimmbewegung von *Dytiscus* und den Portuninen sich aus der phylogenetisch älteren Suchbewegung allmählig herausgebildet hat; während das Schwimmen etwa bei *Hydrophilus* eher aus dem Laufen abzuleiten ist.

Noch eine weitere Bedeutung kann der Boden nicht nur als Grundlage für die Laufbewegung jedes Beines, sondern für die Koordination selbst besitzen. Wenn er rauh genug ist, so dass die Beine nicht ausgleiten, bewirkt er ganz automatisch eine gegenseitige feste Beziehung aller jeweils in der Stemmphase befindlichen Extremitäten; so könnte, wie schon angedeutet, auf mechanischem Wege eine nervöse Koordination vorgetäuscht werden[1].

Diese gegenseitige mechanische Bindung der Beine fällt aber fort, wenn der *Boden* entweder *flüssig*, oder so *glatt* ist, dass er praktisch nur in senkrechter Richtung einen Widerstand darbietet. Beim Laufenlassen eines *Lithobius* auf einer Quecksilberfläche oder auf Spiegelglas zeigt sich nun, dass trotzdem sowohl die Koordination des intakten Tieres, als auch ihre Umstellungen nach Beinverlust sich in der erwarteten Weise einstellen.

Wenn eine Belastung der Beine in vertikaler Richtung für die Laufbewegung selbst erforderlich ist, so wäre mit der Möglichkeit zu rechnen, dass die *Höhe* dieser *Belastung* für die gegenseitige Bewegungskoordination eine Bedeutung hat; denn mit Verminderung der Beinzahl geht notwendig eine Belastungserhöhung der übrigbleibenden Beine Hand in Hand. Auch der Grad der Belastung spielt jedoch für die gegenseitige Bewegungsordnung keine Rolle: das Laufen eines Krebses unter Wasser, wo die Belastung der Beine sich bis auf einen Bruchteil verringert, und an der Luft ist nämlich nicht verschieden. Und künstliche Erhöhung des Körpergewichtes hat bei *Lithobius* keine verändernde Wirkung auf die Koordination.

So bleiben als *bedeutungsvoll für die Beinkoordination* nur noch diejenigen *Erregungen* übrig, *die durch die aktive Bewegung der Beine in Muskeln und Gelenken selbst entstehen.*

Die Frage, welche und wieviele Bestandteile der Extremität für ihren massgebenden Einfluss in der Gesamtordnung wichtig sind, oder, anders ausgedrückt, *welche Teile* man *entfernen* kann, *ohne dass* eine *Koordinationsänderung auftritt*, ist durch v. Buddenbrock, Bethe und Kühl an verschiedenen Arten geprüft worden— zumeist in der einfachen Form einer stückweisen Verkürzung der Extremität. Bei diesem Versuch ist allerdings zu beachten, dass mit einer Verringerung der Zahl der Effektoren und Rezeptoren auch eine Verringerung der bewegten Masse einhergeht, was die Deutung der Ergebnisse komplizieren kann. Zusammenfassend kann man soviel sagen, dass beide dieser Faktoren von Wichtigkeit sein können.

Wird eines der beiden Schwimmbeine von *Dytiscus* allmählig verkürzt, so ist nach Entfernen der äussersten zwei Glieder der fünfgliedrigen Extremität die Koordination noch ganz die alte. Erst bei stückweiser Verkleinerung des dritten

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[1] Das klassische Beispiel einer Koordinationsmöglichkeit auf rein mechanischem Wege ist von Friedländer (1894) beim Regenwurm gefunden worden, den man mitten durchschneiden kann, ohne dass nach Zusammenbinden des Vorder- und Hinterstücks die Koordination zwischen beiden aufgehoben wird (s. auch Holst, 1932, 1933).

Gliedes, des Femur, beginnt das gleichseitige Mittelbein sich an der Bewegung zu beteiligen, indem es im Takt des verkürzten Schwimmbeines mitrudert. Ist die Verkürzung bis zum Ende des ersten Gliedes, der Coxa, vorgeschritten, dann scheidet dieser Beinrest aus der Bewegung aus und das Mittelbein übernimmt die ganze Arbeit.

Etwas anders liegen die Dinge bei den laufenden Formen. Hier ist vor allem die Frage wichtig, ob das verkürzte Bein den Boden noch zu erreichen vermag. Werden einem *Dixippus* die beiden Mittelbeine soweit verkürzt, dass sie den Boden nicht mehr berühren können, so scheiden sie aus der Koordination der übrigen Beine aus und diese verändern ihre gegenseitige Ordnung ebenso wie bei völliger Amputation der Mittelbeine. Wenn den verkürzten Gliedmassen nun ein künstlicher Boden durch Befestigen einer entsprechenden kleinen Fläche am Körper selbst geschaffen wird, laufen die Mittelbeine sofort wieder mit und die Koordination ist der normale Insektengang (v. Buddenbrock, 1921). Das gleiche gilt auch für Krabben; hier können vom sechsgliedrigen Bein vier Glieder amputiert sein— solange es den Boden zu erreichen vermag, ist die Koordination normal. Erreicht der Beinstumpf den Boden nicht mehr, so wird er beim Laufen nicht mitbewegt; dagegen beteiligt er sich bei den Schwimmkrabben wohl an der Schwimmbewegung.

Kühl (1931) hat am Krabbenbein auch einzelne Muskeln durchtrennt, um ihre Bedeutung für die Koordination zu erfahren. Aus seinen Versuchen ergibt sich, dass immer, wenn noch Muskeln erhalten sind, die ein rhythmisches Heben und Senken des Beines oder der Beinspitze bis zum Boden ermöglichen, die normale Koordination bestehen bleibt; auch wenn eine weitere Arbeit vom Beine nicht mehr geleistet werden kann. Wenn aber die Extensoren des Beines soweit ausgeschaltet sind, dass dieses dem Untergrund aufliegt und nicht mehr rhythmisch erhoben werden kann, dann fällt es für die Koordination aus und es erfolgt die zu erwartende Umstellung der übrigen Beine. Bei Verkürzung eines der beiden Schwimmbeine der Portuniden findet eine Koordinationsänderung nicht statt, ebensowenig wie bei gänzlicher Amputation. Die sukzessive Verkürzung hat hier aber eine Beschleunigung der Bewegungsfrequenz des verkürzten Beines zur Folge. Diese ist jedoch nicht nervöser, sondern mechanischer Natur; das verkürzte Bein findet im Wasser geringeren Widerstand; und das Anbringen einer künstlichen Fläche am Beinstummel bewirkt wieder eine entsprechende Verlangsamung des Bewegungsrhythmus.

Wenn aus diesen Verkürzungs- und Ausschaltungsversuchen auch soviel hervorgeht, dass das *Vorhandensein nur eines Teils der Muskeln und Sinnesorgane und die Beweglichkeit nur soweit, als eine rhythmische Hebung und Senkung auf den Boden es erfordert, notwendig* ist, damit das Zentralnervensystem dieses Bein noch als "vollgültig" einordnet, so bleibt eine andere Frage noch offen: sind hier die *Effektoren*, und die Möglichkeit an sie Impulse zu versenden, also die *motorischen Nerven* und *Endplatten* das Wesentliche, oder kommt es auf die *Rezeptoren* an, die dem Zentrum die Ausführung der erteilten Impulse zurückmelden?—möglich wäre beides.

Diese Frage ist aber sofort zu entscheiden, wenn im Versuch zwar die Effektoren

und motorischen Elemente intakt gelassen, die zentripetalen, durch die Bewegung selbst hervorgerufenen Erregungen aber durch teilweises oder völliges *Fixieren des Beines* mehr oder weniger unterbunden werden. Solche Versuche liegen von Buddenbrock, Bethe und Kühl vor.

Fixierung der beiden Mittelbeine am Körper hat bei *Dixippus* zur Folge, dass die übrigen vier Beine in Kreuzgang übergehen, also sich so verhalten, als wenn die fixierten Beine überhaupt entfernt wären. Bei Fixierung einzelner Gelenke des Carcinusbeines durch umgelegte Manschetten zeigt sich, dass das Bein für die Ordnung der übrigen erst ausfällt, wenn die Bewegung soweit behindert ist, dass keine Berührung des Bodens und rhythmische Erhebung von ihm mehr möglich ist. Die *Fixierung eines Gelenkes* ist also ganz *gleichbedeutend mit Durchtrennung* der dieses Gelenk bewegenden *Muskeln*.

Der Schluss, der sich damit ergibt, lautet, dass für die Einordnung eines Beines in die Gesamtkoordination *lediglich* die *zentripetalen*, von den Propriozeptoren in Muskeln und Gelenken bei der Bewegung ausgehenden *Erregungen bedeutungsvoll* sind.

Bei Fixierung einzelner Beine kann es auch zu völliger Störung der Gesamtkoordination kommen; so hört bei Festlegung eines Schwimmbeines von *Dytiscus* das Schwimmen völlig auf, an seine Stelle treten strampelnde Bewegungen der übrigen Beine. In diesem Falle melden die Propriozeptoren nicht "garnichts"— wie bei den vorigen Versuchen—sondern etwas anderes, nämlich die Behinderung der Bewegungsfreiheit, und lösen dadurch Befreiungsversuche der übrigen Beine aus, während die Lokomotion gehemmt wird. .

Die Analyse der für die Beinkoordination wichtigen Faktoren, soweit sie bisher vorgeschritten ist, hat ergeben, dass man *zweierlei unterscheiden* kann: erstens die Erregungen, die für die Laufbewegung jedes einzelnen Beines notwendig sind—sie entstehen durch die Belastung der dem Untergrund aufliegenden Extremität; diese Erregungen sind für die jeweilige gegenseitige Ordnung der Beine bedeutungslos. Zweitens die Erregungen, die bei der Bewegung des Beines selbst in seinen Muskeln und Gelenken entstehen—sie sind der für die gegenseitige Koordination der Beine beim Laufen wichtige Faktor. Mit diesen beiden Tatsachen haben die theoretischen Vorstellungen, die man sich über die Natur des Koordinationsprinzips bilden kann, zu rechnen. Bevor wir aber auf diese Deutungen selbst eingehen, sind noch einige Arthropoden zu besprechen, deren Koordinationsform von den bisherigen wesentlich abweicht.

VI. ANDERE KOORDINATIONSPRINZIPIEN.

Die Koordination der bisher besprochenen Arten zeichnet sich dadurch aus, dass die Bedingungen, die sie sowohl zustandekommen lassen, als auch zu verändern vermögen, im Körper des Tieres selbst gelegen sind; wechselnde äussere Laufbedingungen, wie Unregelmässigkeiten des Bodens, können diese Ordnung höchstens stören, aber nicht umändern. Wir wollen diesen Koordinationstyp den "endoplastischen" nennen, im Gegensatz zum jetzt zu besprechenden "exoplastischen"

Typ, bei dem die Beinordnung sich dauernd in weiten Grenzen wandelt und das Koordinationsprinzip lediglich darauf eingestellt ist, eine fortwährende Anpassung an die wechselnden Unebenheiten des Bodens zu ermöglichen. Dieser durch Holst (1934 *a*, *d*) aufgefundene Typ ist bisher nur von einigen *Chilopoden* oder Hundert-füssern (*Geophilus longicornis*, *Geophilus ferrugiulus*, *Cryptops hortensis*) bekannt; seine Analyse ist bedeutend einfacher, als die des endoplastischen Typs.

Wir halten uns zunächst an *Geophilus*, einen schlanken, wurmähnlichen Hundertfuss von inkonstanter Segmentzahl (40–80 Segm.). Die Beinordnung des laufenden *Geophilus* zeichnet sich durch folgende Eigenheiten aus: eine bestimmte Phasenbeziehung zwischen dem rechten und linken Bein eines Segments fehlt völlig. Beim Laufen auf ebenem Boden können die Beine sowohl synchron, als auch alternierend oder in jeder anderen Ordnung aufgesetzt werden, ohne dass eine dieser Möglichkeiten vorherrscht. Die Ordnung der hintereinander folgenden
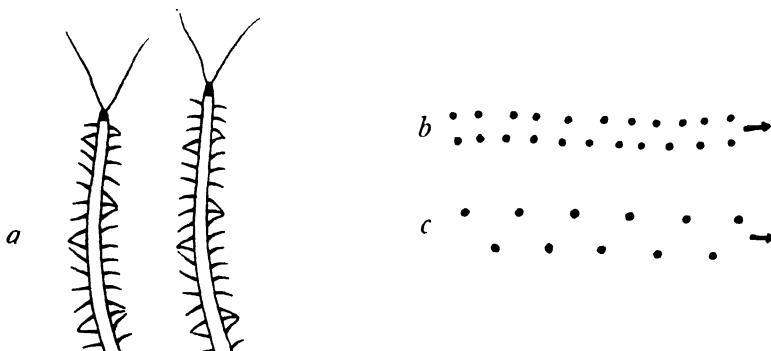
Abb. 6. *a*, Vorderes Drittel eines laufenden *Geophilus* (nach Filmen von Holst); *b*, *c*, Die von laufenden Chilopoden hinterlassenen "Fusstapfen": *b*, *Geophilus* über ebene Fläche; *c*. *Cryptops* über ebene Fläche gelaufen. (Schematisch nach Laufspuren über berussten Untergrund, von Holst.)

Beine einer Seite ist derart, dass jedes nachfolgende Bein um einen in seiner Grösse sehr wechselnden Bruchteil einer Schrittphase dem davorgelegenen Bein folgt; und zwar tritt jedes nachfolgende Bein immer genau auf den gleichen Punkt des Bodens auf, den das vordere Bein soeben verlassen hat. Es benutzen also sämtliche Beine einer Seite den gleichen "Fusstapfen," d. h. den Berührungspunkt, den das erste Bein gewählt hat, wie Abb. 6 *a* es zeigt. Lässt man *Geophilus* über eine berusste Fläche laufen, so zeichnen sich diese Fusstapfen durch deren jeden alle Beine einer Seite hindurchgegangen sind, auf. Eine solche Spur zeigt Abb. 6 *b*; man sieht, dass keine feste Beziehung zwischen rechtem und linkem Fusstapfen besteht, und dass ferner der Abstand zwischen einem und dem nächsten Fusstapfen inkonstant ist. Die Beinordnung ist auch hier, wie bei *Lithobius*, wellenförmig, nur dass die "Wellen" über den Körper nicht von hinten nach vorn, sondern von vorne nach hinten ver-laufen, im Verhältnis zum Substrat aber an der gleichen Stelle verharren.

Der Sinn dieses Koordinationsprinzips erhellt sofort, wenn man das Laufen über unebenes Gelände betrachtet. Hier werden sowohl das Phasenverhältnis zwischen

den rechten und linken Beinen, als auch die Abstände von einem zum nächsten Fusstapfen allein durch die Gestalt der jeweiligen Bodenerhebungen bestimmt. Das vorderste Beinpaar des laufenden Tieres macht dauernd Suchbewegungen, und sobald ein Bein den Boden berührt hat, macht es augenblicklich einen Schritt und leitet nun das nächste Bein auf den gleichen Berührungspunkt usf. So ist die *grösstmöglichste Anpassung an die Bodenverhältnisse* erreicht und jedes Bein findet stets seinen Fusspunkt; das Tier gleitet nur über die jeweils höchsten Erhebungen des Bodens ziemlich gradlinig dahin.

Das Koordinationsprinzip von *Cryptops*, einem Chilopoden von 20 Segmenten Länge stimmt mit dem von *Geophilus* überein, mit der Abweichung, dass auf ebenem Gelände sich hier wohl ein bestimmtes Phasenverhältnis zwischen rechts und links, nämlich das der Alternation, einstellt und dass die Fusstapfenabstände in viel geringeren Grenzen variieren. Eine Laufspur auf ebenem Boden zeigt Abb. 6 *c*.

Der Versuch der Beinamputation hat bei dem exoplastischen Typ keine Änderung der Koordination zur Folge, wie beim endoplastischen Koordinationstyp. Das einzige, was sich erreichen lässt, ist eine Unterbrechung der nervösen Bindung. Das Fehlen eines einzelnen Beines aus der Mitte des Körpers stört die feine nervöse Beziehung der Beine einer Seite nicht merklich; nach Amputation von zwei Beinen vermag das dritte und damit alle nachfolgenden den Fusspunkt der vorderen Beine nur ungenau, nach Amputation von drei (aufeinanderfolgenden) Beinen überhaupt nicht mehr zu finden. Die hinter der Lücke gelegenen Beine benutzen nun neue Fusstapfen. Bei *Cryptops* laufen in diesem Falle die hinteren meist in einem schnelleren Rhythmus als die vorderen und damit alle übrigen Gliedmassen.

Zur Erhaltung der Koordination aufeinanderfolgender Beine ist eine längere Segmentreihe nicht erforderlich: auch *Stücke von nur zwei Segmenten*, die man aus der Körpermitte herausschneidet, und die noch selbstständig zu laufen vermögen, zeigen die normale Koordination; das hintere Bein tritt in die Fusstapfen des vorderen. Bei *Cryptops* verschwindet dabei allerdings die regelmässige Alternation zwischen rechten und linken Beinen; diese Funktion ist an die Zusammenarbeit einer grösseren Segmentzahl gebunden.

Der letzte, durch Bethe und seine Mitarbeiter (1931 *c*, 1933) studierte Koordinationstyp, der sich zwanglos weder in den exo- noch in den endoplastischen Typ einfügt, findet sich bei Tausendfüssern (Diplopoden), von denen die Gattung *Iulus* als Untersuchungsobjekt diente. Die Diplopoden zeigen die Besonderheit, dass je zwei Segmente miteinander verschmolzen sind, sodass deren jedes vier Beine trägt und alle Beinpaare, gegen 200, sehr dicht zusammenrücken.

Die Anordnung der Beine eines *Iulus* beim Laufen ist wie bei den Chilopoden wellenförmig (Abb. 7). Die Koordination von Bein zu Bein ist dabei ähnlich der bei *Lithobius*: die "Wellen" wandern von hinten nach vorn über den Körper. Die einander gegenüberliegenden Beine laufen aber hier stets synchron. Die Länge der Wellen, also der Phasenabstand von Bein zu Bein, schwankt nur in engen Grenzen; und zwar pflegt jede Welle in der Mitte des Körpers etwas an Länge zu- und weiter vorne wieder abzunehmen.

Bei einseitiger Amputation einer Anzahl von Beinen (15–20) aus der Körpermitte wird die Ordnung der übrigbleibenden kaum geändert. Es tritt lediglich eine geringe, sich immer wieder ausgleichende Rhythmusbeschleunigung der gleichseitigen Beine hinter der Lücke auf. Werden die entsprechenden Beine der anderen Seite ebenfalls entfernt, so weicht der Bewegungsrhythmus der vorderen und der hinteren Beingruppe voneinander ab; und zwar bewegen sich die Beine hinter der Lücke wie beim entsprechenden Versuch mit *Cryptops*, anscheinend gegenüber den vorderen beschleunigt.

Eine weitgehende *Verkürzung* bis auf nur wenige Segmente ist bei *Iulus* nicht mit Erfolg ausführbar; nur längere Kopfstücke vermögen noch gut selbständig zu laufen. Mit fortschreitender Verkürzung von hinten her ändert sich die Koordination sukzessive: die Phasenabstände verringern sich. Halbierung z. B. hat eine Verringerung der Phasenabstände und eine Beschleunigung des Laufrhythmus um etwa 15–25 % zur Folge. Diese Änderung glaubt Bethe auf die Verkleinerung des nervösen Areals, in dem sich die rhythmische Erregung ausbreitet, zurückführen zu können.
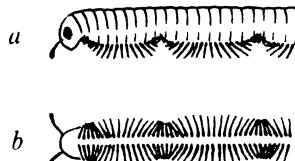
Abb. 7. Vorderes Drittel eines laufenden *Iulus*, *a* von der Seite, *b* von unten gesehen (nach Filmen von Bethe).

Eine Anpassung der Beine an Unebenheiten des Untergrundes findet bei *Iulus* in erster Linie insofern statt, als die Beine bei Bodenerhebungen mehr gespreizt, bei Vertiefungen weiter herabgebeugt werden.

Wenn man, soweit es nach den vorliegenden Versuchen möglich ist, diesen letzten, gleichsam aberranten Typ mit den beiden anderen vergleicht, so fällt vor allem auf, wie gering die Koordinationsänderungen sind, die man hier durch Ändern der inneren und äusseren Bedingungen erzielt. Dieser Koordinationstyp ist verhältnismässig starr und fest gefügt und damit für unser Problem von geringerem Interesse.

VII. PHYSIOLOGISCHE HYPOTHESEN ZUR DEUTUNG DER KOORDINATION.

Kehren wir zum zuerst besprochenen *endoplastischen Typ* zurück. Die Regeln, die wir dort für die Beinordnung beim Laufen kennenlernten, entheben uns noch nicht der Notwendigkeit, genauere Vorstellungen über ihr Zustandekommen zu bilden. Diese Koordinationsregeln verbinden nur bestimmte Ausgangsbedingungen mit einem bestimmten Endresultat—sie sagen aber über dessen Zustandekommen ebensowenig aus, wie etwa die Mendelschen Regeln der Vererbung über die Faktoren, die bei ihrer Realisierung im einzelnen wirksam sind.

Am meisten hat Bethe sich, im Rahmen seiner "Plastizitätslehre" (1931 *a*, 1931 *b*), um eine Deutung der Erscheinungen bemüht. Bethe vergleicht die koordinierende Funktion des Zentralnervensystems mit dem mechanischen Modell einer "gleitenden Koppelung," d. h. einem Hebelsystem, bei dem keine feste, sondern eine gleitende, sich je nach den jedem Hebel entgegenstehenden Widerständen verändernde, gegenseitige "Koordination" besteht.

Für viele Fälle lässt sich nach Bethe die Vorstellung von Uexkülls (1921) von einem "Erregungstal" im Bauchmark, dem die Erregung zufliesst, heranziehen. Am leichtesten gelingt das für das Schwimmen von *Dytiscus* sowie den Lagereflex von *Carcinus*. Das "Tal" liegt in beiden Fällen am Hinterende des Tieres, denn allein das letzte Beinpaar ist normaler Weise tätig. Wird dieses nun entfernt, so ist von den übrigen Ganglien das vorletzte das Erregbarste, hierhin fliesst jetzt die Erregung, d. h. das vorletzte Beinpaar tritt in Funktion, usw. Indessen sind auch andere Vorstellungen möglich, z. B. die, dass das letzte Beinpaar bei seiner Tätigkeit die übrigen Beine hemmt; nach seiner Entfernung fällt diese Hemmung fort.

Auch für die Koordination beim Laufen hat Bethe (1931 *c*, 1933) an *Iulus* die gleiche Vorstellung eines Erregungstales verwendet. Er nimmt, ähnlich wie auch v. Buddenbrock (1921) es für *Dixippus* versucht hat, an, dass die einzelnen *Ganglien verschieden stark erregbar* sind und dass im Rhythmus der Beinbewegung *Erregungen durch das Nervensystem* laufen, die vom erregbarsten Ganglion ihren Ausgang nehmen und jeweils den Anstoss zu einer Bewegung geben. Bei *Iulus* ist, so vermutet Bethe, das letzte Ganglion das erregbarste, weil hier die Bewegungswellen ihren Ausgang nehmen, und von hier werden die rhythmischen Erregungen, vielleicht mit einem gewissen Dekrement, nach vorne gesandt.

Man kann indessen die Erscheinungen hier auch anders deuten; meiner Ansicht nach besagt der Verlauf der "Wellen" nichts über einen ihnen entsprechenden Erregungsverlauf im Bauchmark, sondern er ist nur der Ausdruck eines bestimmten gegenseitigen Phasenverhältnisses (s. S. 235 und Holst, 1934 *c*).

Auch eine andere Erscheinung, die Uexküll (1921, 1929) gefunden hat, und die als *Dehnungsregel* bezeichnet wird, hat Bethe zur Deutung herangezogen. Diese Regel besagt, dass in vielen Fällen die Bewegung eines Organes, z. B. Beines, nicht allein vom Zentrum her, sondern durch die im Moment gerade eingenommene Lage des Beines selbst bestimmt wird; und zwar in der Weise dass die gerade gedehnten Muskeln sich verkürzen, die kontrahierten erschlaffen. Wenn man nun (mit Bethe, 1931) annimmt, dass ein Bewegungsanstoss immer dann durch das Bauchmark des laufenden Tieres zirkuliert, wenn die Beine gerade eine extreme Stellung einnehmen, so wäre damit eine Aufrechterhaltung des bestehenden Ganges gewährleistet. Aber auch die Dehnungsregel vermag, wie Bethe selbst sagt, nicht zu erklären, wie die Beine zunächst aus der Ruhelage in die charakteristische gegenseitige Ordnung gebracht werden und vor allem, wie sie dieser Ordnung nach mechanischen Störungen immer wieder zupendeln. Auch die Koordinationsumstellungen nach Beinverlust bleiben unverständlich.

Wir können das Koordinationsproblem vielleicht am klarsten fassen, wenn wir im Gedanken zweierlei von einander trennen: erstens die Laufbewegung eines jeden

einzelnen Beines, und zweitens das gegenseitige in Beziehung treten aller Beine. Die Lauftätigkeit jedes Beines ist entweder reflektorischer Natur (das Bein erzeugt bei jedem Schritt selbst den Reiz zum nächsten Schritt) oder, nach Holst (1934 c), automatischer Natur (jedes Bein besitzt im Ganglion einen eigenen, automatisch arbeitenden Motor). Die gegenseitige Koordination ist reflektorischer Natur: wir sahen, dass das Bein nur, wenn es sich aktiv mitbewegt, in die Ordnung der übrigen Beine eingefügt wird und deren Koordination mitbestimmt; wird es unbeweglich gemacht, so ist das gleichbedeutend mit Amputation. Die (auf S. 240 und folgende) besprochenen Koordinationsregeln geben uns weiterhin Aufschluss, dass zwischen der Zahl der bewegten Beine und ihrem Phasenabstand, also der gegenseitigen anatomischen Lagerung, quantitative Beziehungen bestehen; je mehr Beine, umso geringer der Phasenabstand von Bein zu Bein. Auf Grund dieser Tatsache darf man sich hypothetisch vorstellen, dass die Koordination ein reflektorischer Vorgang ist, der überhaupt erst durch die Bewegung ausgelöst wird; beginnt das Tier zu laufen, so sendet jedes tätige Bein eine gewisse zentripetale Erregung aus, die sich im Bauchmark verteilt. Diese Erregung summiert sich mit der Zahl der laufenden Beine und je nach der Höhe der resultierenden Gesamtsumme sendet nun das Bauchmark dauernd eine bestimmte Erregung in jedes Bein zurück, die eine gegenseitige Lagerung der Beine in einem gewissen Phasenabstand bewirkt. Dieser Prozess ist meiner Meinung nach nur deshalb schwierig bildlich vorzustellen, weil die Beine nicht in der eingenommenen Koordinationslage zur Ruhe kommen, sondern auf diese gegenseitige Ordnung sich die zirkulierende Laufbewegung gleichsam noch aufsetzt.

Auf Grund dieser Vorstellung haben wir es nun bei den Koordinationsumstellungen des endoplastischen Typs nach Beinverlust nicht mit einer besonderen, neuen Eigenschaft des Zentralnervensystems zu tun, sondern mit den gleichen Faktoren, die auch schon bei der normalen Koordination des intakten Tieres wirksam sind. *Die normale Ordnung* und die *neue, nach Beinamputation auftretende Ordnung beruht* meines Erachtens *auf ein und demselben Grundprinzip*.

Weniger schwierig als die Bewegungskoordination ist nach meiner Meinung die *Haltung* und ihre Änderung nach Beinamputation, wie sie am deutlichsten bei *Opilio* (Abb. 5) auftritt, zu deuten. Man weiss, insbesondere von den höheren Wirbeltieren und dem Menschen, dass die Stellung der Extremitäten, auf denen der Körper lastet, durch die in ihnen selbst gelegenen Rezeptoren dauernd kontrolliert und einer Korrektur unterworfen wird. Das gleiche dürfte auch hier gelten, und zwar ist dabei in diesem Falle in erster Linie an die Stärke der Belastung des einzelnen Beines zu denken. Nach Entfernung eines oder mehrerer Beine ist die Last, die deren Nachbarn tragen müssen, vergrössert, und unter Umständen, wenn bereits eine Neigung zum Umfallen besteht, die der gegenüberliegenden Beine verringert.

Wenn die Annahme gemacht werden darf, dass ein solches Ungleichgewicht der Erregungen eine derartige Umstellung der Beine zur Folge hat, dass die Last wieder möglichst gleichmässig verteilt wird, dann braucht man wohl auch für die Haltungsänderungen nach Beinamputation keine andern Faktoren zur

heranzuziehen, als auch bei der Haltungsregelung des intakten Tieres wirksam sind.

Die physiologische Deutung des *exoplastischen Typs* dürfte vom folgenden Versuch aus am leichtesten gelingen. Wie bereits erwähnt, vermögen kleine herausgeschnittene Stücke von *Geophilus* und *Cryptops* noch wohlkoordiniert zu laufen. Ein solches Tierstück kann man nun mit der Pinzette ergreifen und über den Boden in wechselnder Geschwindigkeit dahinführen, auch einen Augenblick anhalten usw., wobei alle Beine in der aufgezwungenen Geschwindigkeit und in völliger Ordnung, jedes in die Fusstapfen seines Vordermannes hineintretend, mitlaufen. Nun überträgt sich hierbei die dem Tierstück diktierte Bewegung lediglich auf die gerade am Boden, in der Stemmphase, befindlichen Beine direkt auf mechanischem Wege. Dass alle Beine einheitlich reagieren, bedeutet also, dass aus den Propriozeptoren jedes in der Stemmphase befindlichen Beines Erregungen ausgehen müssen, die zu der Reihe der unmittelbar folgenden in der Schwingphase begriffenen Beine gelangen und deren Lauftätigkeit je nachdem hemmen oder beschleunigen. An die Stelle der im Versuch mechanisch aufgezwungenen verschiedenen Geschwindigkeit tritt beim frei beweglichen Tiere der sich auf die Laufgeschwindigkeit der Stemmbeine auswirkende verschiedene Erregungszustand.

Nach dieser Darstellung weicht der exoplastische Koordinationstyp sehr wesentlich vom endoplastischen ab; dafür entspricht er weitgehend den Verhältnissen, wie sie nach den neueren Ergebnissen bei höheren Würmern, etwa dem Regenwurm (Holst, 1932, 1933) vorliegen, mit dem auch sonst morphologische und besonders physiologische Ähnlichkeiten bestehen.

VIII. DIE BEDEUTUNG DER HÖHEREN ZENTREN.

Aus dem umfangreichen Kapitel, welche Funktionen den höheren Zentren, dem Oberschlundganglion oder Hirn und dem Unterschlundganglion bei den Arthropoden zukommen, können wir hier nur das auswählen und kurz behandeln, was mit unserem Thema im Zusammenhang steht, oder was wenigstens für das Verständnis der hier entwickelten Vorstellungen von Bedeutung ist.

So sind vor allem *zwei Fragen* von Interesse: Kommt die Koordination der Beinbewegung in höheren Zentren oder in den Bauchganglien selbst zustande? Und: In welcher Form können die höheren Zentren überhaupt Bewegung und Haltung des Körpers beeinflussen?

Die erste Frage ist für den exoplastischen Typ am leichtesten zu beantworten: Wenn kleine Teilstücke von *Geophilus* oder *Cryptops* noch normal laufen, so beweist das die Unabhängigkeit der Beinordnung von im Kopf gelegenen Zentren. Ähnlich liegen die Dinge bei *Iulus*, wo kopflose Stücke sich zwar ungeschickt, aber mit normaler Beinordnung fortbewegen.

Für viele andere Arthropoden hat man dagegen früher, auf Grund der Tatsache, dass sie nach Exstirpation von Ober- und Unterschlundganglion keine Laufbewegungen mehr ausführen, auf besondere Bewegungs- oder *Koordinationszentren* in diesen Ganglien geschlossen. Dieser Schluss ist in neuerer Zeit einer Revision

unterzogen worden—denn es scheint, als wenn hier die koordinierende Funktion mit einer ganz anderen Eigenschaft, auf die wir gleich zu sprechen kommen, verwechselt worden ist.

Es gelingt nämlich bei fast allen Formen, die nach Verlust beider Schlund-ganglien nicht mehr freiwillig laufen, doch, die Tiere durch Reize verschiedener Art dazu zu bringen, dass sie eine kürzere oder längere Strecke zurücklegen; dabei ist die Beinkoordination gewöhnlich ebenso oder sehr ähnlich wie vor der Operation. An *Dytiscus* gelang es Bethe (1930 *b*) sogar, auch beim schlundganglionlosen Tier alle nach Beinamputation auftretenden Koordinationsumstellungen der Schwimm-bewegungen nachzuweisen. Aus diesem Grunde lehnt Bethe (1931) heute die Ansicht ab, dass die Koordination in bestimmten Zentren lokalisiert sei und vertritt die Meinung, dass für das Zusammenspiel aller Teile jeder Teil von Bedeutung ist und dass die jeweilige Koordination als Leistung des gesamten noch vorhandenen Zentralnervensystems und der gesamten Peripherie aufgefasst werden müsse.

Die Tatsache, dass nach Entfernung der Schlundganglien die spontane Loko-motion bei zahlreichen Formen—fast allen mit Ausnahme der schlankeren, mehr homonom segmentierten Typen—erlischt, wird durch v. Buddenbrock (1928) im Rahmen seiner Theorie der "Energiezentren" so gedeutet, dass wir es hier mit einem derartigen "Energiezentrum" im Unterschlundganglion zu tun haben, welches den Erregungszustand des ganzen Bauchmarks beherrscht und erhöht, indem es selbst Erregung sammelt, speichert und wieder aussendet. Als ein derartiges Zentrum kommt nur das Unterschlundganglion in Frage, denn nach blosser Enthirnung ist die Lauftätigkeit meist kaum gestört, bisweilen sogar gesteigert (Jawlowski, 1929). Diese Auffassung des "Erregungszentrums" allein aus der Tatsache der fehlenden freiwilligen Beweglichkeit abzuleiten, ist vielleicht gewagt; aber es sprechen meines Erachtens noch weitere Gründe für die Brauchbarkeit des gewählten Bildes: einmal, dass es gelingt, das fehlende Zentrum durch bestimmte oder sogar beliebige, auf anderem Wege zugeführte Erregung zu ersetzen. Und dann die von vielen Untersuchern (Bethe, 1897; Jordan, 1910; Kopec, 1919; Holst, 1934 *d*; u.a.) an verschiedenen Arthropoden gemachte Beobachtung, dass beim Fehlen der Schlundganglien die Reizschwellen für alle möglichen Reflexe wesentlich erniedrigt, also diese Bewegungen leichter auslösbar sind.

Man könnte es sich als Gleichnis so vorstellen: Von jeder auf einen Reiz hin entstehenden Erregung wird ein Teil in das "Energiezentrum" abgeleitet, der andere Teil für den diesem Reiz zugeordneten Reflex verwandt. Sind nun die Bahnen zum "Energiezentrum" durchtrennt, so wird die ganze Erregung für den Reflex verbraucht, seine Erregbarkeit steigt also. Die im "Energiezentrum" gesammelte Erregung wird nun (vielleicht nur unter anderem) für solche Funk-tionen "verbraucht," die nur auf der Basis eines erhöhten, oder wenn man will, veränderten Erregungszustandes ablaufen—und eine solche Funktion ist offenbar die Laufbewegung[1]. (Dass es Tätigkeiten gibt, die bei niederem und andere, die

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[1] Die vom Energiezentrum ausgehende Erregung verläuft (bei Lepidopterenlarven nach Holst, 1934 *d*) durch zwei parallel laufende Bahnen, die in jedem Ganglion der Bauchkette ganz spärliche Überkreuzungen besitzen.

erst bei höherem, allgemeinem Erregungszustand in Erscheinung treten, dafür gibt es in der Biologie mancherlei Beispiele, und dass gerade das Laufen zu den letzteren gehören soll, darf man vielleicht vermuten.)

Bei Formen wie *Geophilus*, wo Teilstücke ebenso schnell umherlaufen, wie ganze Tiere, kommt noch jedem Ganglion der Bauchkette die Funktion der Erregungssammlung zu—das ist der primitivere Zustand, wie man ihn auch beim Regenwurm (*Lumbricus*) findet. Zwischen diesem und dem anderen Extrem gibt es Übergänge, wie etwa *Cryptops*, bei dem Teilstücke merklich langsamer laufen und ungereizt immer schnell wieder zum Stehen kommen (Holst, 1934 *d*).

Dieser ganze Versuch einer Deutung der Erscheinungen hat vor anderen den Vorteil der Einfachheit voraus; ob er der Wirklichkeit damit näher kommt, bleibt vorläufig dahingestellt. Jedenfalls sind auch andere Vorstellungen möglich; so hat man vielfach die erhöhte Reflexerregbarkeit nach Entfernung des Hirns bzw. beider Schlundganglien mit dem Ausfall eines besonderen Hemmungszentrums zu erklären gesucht. In etwas modifizierter Weise nimmt Herter (1932) "Aktivitätsverminderungsbahnen" an, die gewisse aktivitätserhöhende Faktoren vom Bauchmark in die Schlundganglien ableiten, wo sie vernichtet werden. Umgekehrt müsste dann aber die fehlende Spontaneität vieler schlundganglienloser Tiere den Schluss auf besondere Erregungs- oder Bewegungszentren rechtfertigen. Für die Annahme solcher getrennter hemmender und erregender Faktoren spricht die Erscheinung, dass es Formen gibt, bei denen die erhöhte Reflexerregbarkeit schon nach Entfernen des Hirns deutlich auftritt, während die Lauftätigkeit dann nur vermindert ist und erst nach Ausschaltung des Unterschlundganglions ganz erlöscht.

Während so die Hauptfunktion der höheren Zentren darin besteht, den allgemeinen Aktivitäts- oder Tätigkeitszustand dés Gesamtnervensystems zu beherrschen, besitzen sie—und zwar vor allem das *Hirn* als Zentralstelle für die an der Orientierung beteiligten Sinnesorgane—insbesondere auch die *Fähigkeit*, die *Koordination von Haltung und Bewegung* soweit *zu verändern*, als es die Änderung der allgemeinen Laufrichtung erfordert. Diese Fähigkeit tritt vielfach bei asymmetrischen Eingriffen an den Rezeptoren, z. B. einseitiger Blendung, ganz regelmässig jedoch nach einseitiger Entfernung oder Ausschaltung des Hirns zutage. *Verlust der rechten Hirnhälfte* beispielsweise hat stets zur Folge, dass das Tier beim Laufen fortdauernd *Kreise nach links* herum beschreibt.

Je nach den Lokomotionsmitteln wird das auf verschiedene Weise erreicht: Bei Raupen (Lepidopterenlarven) durch Linkskrümmung, bei Krabben durch veränderte Bewegungsrichtung der rechten und linken Beine—die linken arbeiten mehr nach seitlich, die rechten nach vorne—bei Hundertfüssern (Chilopoden) durch Krümmung des Körpers und verschiedene Exkursionsweite der Beine beider Seiten.

Diese Bewegungsasymmetrien sind seit Treviranus (1832) von vielen Forschern (so Bethe, 1897 *b*; Jordan, 1910; Kopec, 1919; Segaar, 1929, in neuester Zeit eingehend von Herter, 1932) beschrieben worden. Die ältere Deutung dieser Erscheinung ging dahin, dass von jeder Hirnhälfte gewisse Einflüsse auf die gleichseitige Körperhälfte ausgehen, die darin bestehen, den Tonus aufrecht zu erhalten (Raupen, Hundertfüsser) und der Bewegung der Beine eine bestimmte

(bei Krabben z. B. seitliche) Richtung zu geben; nach einseitigem Ausfall dieser Einflüsse tritt dann eine *Dissonanz zwischen rechts und links* ein, die zwangsläufig zum Kreisgang führt.

In neuerer Zeit hat sich diese Deutung gewandelt (Alverdes, 1925; Baldus, 1927; Kühl, 1933; Holst, 1934 *b*). Baldus und Kühl zeigten nämlich, dass das Tier nach einseitiger Enthirnung auf beiden Körperseiten in gleicher Weise auf Kreisgang eingestellt ist, und dass es nach Amputation von Beinen (Insekten, Krabben) Haltung und Bewegung der übrigbleibenden immer so abändert, dass eine Drehung nach der Seite der intakten Hirnhälfte erfolgt. So vermag *Carcinus* nach Amputation aller Beine der Defektseite doch noch eine Drehung nach der Gegenseite auszuführen, wobei er beide Scheren als Hilfswerkzeuge verwendet, wie Abb. 8 schematisch zeigt.

Sprechen schon diese Befunde deutlich gegen den Ausfall einer bestimmten Funktion auf einer Körperseite und für eine *harmonische Gesamtumstellung*, so wird diese Meinung weiter gesichert durch Beobachtungen Holsts an einer Raupe
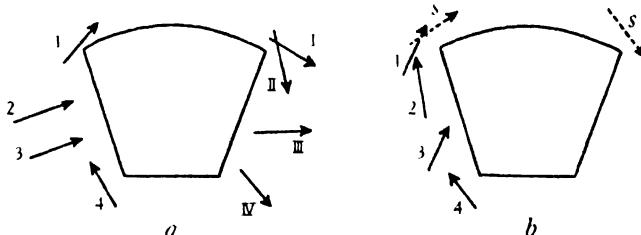

Abb. 8. Schema, das die Bewegungsrichtung der Beine (während ihrer Stemmphase) bei *Carcinus* angibt: *a*, rechts enthirntes, sonst intaktes Tier; *b*, rechts enthirntes Tier nach Entfernung der vier rechten Beine; hier werden auch die Scheren (*S*) zu Hilfe genommen; die Beine sind links mit arabischen, rechts mit römischen Zahlen bezeichnet. (Nach Kühl, etwas verändert.)

(*Hepialis*), einem Tier, das ebensogut vorwärts wie rückwärts zu laufen vermag. Die rechts enthirnte Raupe ist beim Vorwärtslaufen nach links gekrümmt, beschreibt eine Drehung nach links; kriecht sie aber rückwärts, so wird der Körper leicht nach rechts herumgebogen, wodurch wiederum eine Drehung des laufenden Tieres nach links erreicht wird. Es ist also *nicht eine bestimmte*, auf Ausfall gewisser Zentren beruhende *Asymmetrie*, sondern die Drehtendenz nach der intakten Seite die *primäre Ursache* der—je nach den sonstigen Umständen verschiedenen—Haltungsänderung. Der Grund für diese Drehtendenz dürfte der einseitige Ausfall der Rezeptoren, die der Orientierung dienen, sein; was, nach Uexkülls Ausdrucksweise, eine Verschiebung des "Merkraumes" und damit auch eine Verschiebung der Symmetrieebene des "Wirkraumes" zur Folge hat (Kühl).

Wenn der Einfluss, den das Hirn auf den Körper zu nehmen vermag, indem es die Bewegungsrichtung der Beine ändert, das tonische Gleichgewicht verschiebt usw. auch vielleicht von qualitativ verschiedener Form erscheint, so lassen doch die zum Schluss zu besprechenden Versuche die Möglichkeit zu, das es sich nur um *quantitativ abgestufte Erregung* handelt.

Jordan (1910, 1929, bestätigt durch Herter, 1932) brachte an Krabben nach

Entfernung einer, z. B. der rechten Hirnhälfte Elektroden an der nunmehr frei endigenden rechten Schlundkommissur an. Das Tier läuft in Kreisen nach links; wird die rechte Kommissur nun elektrisch gereizt, so wird bei schwächerem Reiz der Linksgang mehr oder minder abgemildert, bei genügend starker Reizung sogar in Rechtsgang verwandelt[1]. Es gelingt also, die *fehlende Wirkung der einen Hirnhälfte* durch Zuführen eines an ihre Stelle tretenden elektrischen Reizes nach Belieben *quantitativ zu ersetzen*. Man darf daher auch beim intakten Hirn mit einer von ihm ausgehenden verschiedenen Erregungs-"menge" rechnen[1].

Dass es sich um Erregungsquantitäten handelt, darauf deutet auch der folgende einfache Versuch Holsts (1934 *b*) hin. Bei den Hundertfüssern *Lithobius* und *Cryptops* sind die nach einseitiger Enthirnung gelaufenen Kreise regelmässig und von bestimmter Grösse. Wird nun diesen Tieren das Bauchmark von hinten her sukzessive verkürzt, so nimmt die tonische und motorische Asymmetrie im Vordertier immer mehr zu: die Kreise werden immer kleiner und besitzen z. B. bei Verkürzung um die Hälfte nur noch einen weniger als halb so grossen Durchmesser. Holst schliesst daraus, dass "*eine umgekehrte funktionale Beziehung besteht zwischen der quantitativen Wirkung eines Zentrums und der Grösse des übrigen zentralen Bereiches*, in dem die von ihm ausgehende Erregung sich ausbreitet." Man kann es sich bildlich so vorstellen, dass eine bestimmte, den Tonus des Körpers und die Schrittweite der Beine in allen Segmenten verschiebende Erregungsmenge, die aus der intakten Hirnhälfte kommt, sich das eine Mal auf viele, das andere Mal auf wenige Ganglien verteilt; im ersten Fall ist der Anteil jedes Ganglions an dieser Erregung geringer, im zweiten entsprechend höher.

Es ist das eine ähnliche Erklärung, wie sie wohl zuerst für die Erhöhung der Reflexerregbarkeit beim Frosch nach Ausschaltung des Hirns von Herzen (1864) und später auch von Anderen für ähnliche Erscheinungen versucht worden ist (Bethe, 1931 *c*, 1933; Thorner, 1932). Auch die bei Besprechung des "Energiezentrums" (S. 254) vorgebrachten Argumente gehen z. T. in die gleiche Richtung. Man muss sich indessen dabei bewusst bleiben, dass solche Deutungen mit einer bis jetzt noch nicht bewiesenen stillschweigenden Voraussetzung rechnen— nämlich der, dass eine Erregung, die ihren Weg durch eine bestimmte Leitungsbahn nimmt, nach Unterbrechung derselben nicht diese Bahn benutzt und damit erfolglos verpufft, sondern dass sie dann andere Wege einschlägt und sich *an anderer Stelle* auswirken kann.

IX. AUFTRETEN NEUARTIGER LOKOMOTIONSWEISEN NACH VÖLLIGEM BEINVERLUST.

Wenn ein Krebs unter besonderen Umständen die Scheren bei der Fortbewegung zu Hilfe nimmt, so liegt das noch im Rahmen der normalen Lokomotion. Bei vielen Arthropoden findet man aber, dass nach Entfernung oder beim Untauglichwerden der Laufextremitäten Lokomotionsweisen auftreten, die als völlig neuartig

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[1] Diese Erregung wird vom Hirn, nach Versuchen an Lepidopterenlarven (Holst, 1934 *d*) auf zwei Bahnen, die im Unterschlundganglion sehr weitgehend, in den übrigen Ganglien aber überhaupt nicht überkreuzen, ins Bauchmark transportiert.

erscheinen und in denen die Tiere es oft zu bewundernswerter Fertigkeit bringen. Solche Beispiele sind von Bethe (1930), Kühl (1932) und Holst (1934 *a*) beschrieben worden.

Werden einem *Opilio* alle acht Beine entfernt, so benutzt das Tier mit Geschick die kleinen Pedipalpen, die normaler Weise den Boden überhaupt nicht berühren, zur Fortbewegung, indem es sie alternierend dem Boden aufsetzt und sich so vorwärtszieht (Bethe). *Lithobius* verwendet nach Verlust aller Beine die kräftigen Beisszangen; er erfasst damit Gegenstände der Umgebung und zieht sich dann mit einem Ruck heran. In dieser Fähigkeit erreichen manche Tiere nur eine geringe, einzelne aber eine hohe Vollkommenheit (Holst). Der beinlose *Cryptops* verwendet nicht die Zangen, sondern er sucht sich nach Schlangenart um Erhebungen herumzuwinden; ausserdem gehen dabei Verkürzungswellen von vorne nach hinten über den Körper. Die Fähigkeit sich so fortzubewegen war bei einem über ein halbes Jahr beobachteten Tier zum Schluss eine erstaunliche (Holst).

Der beinlose *Hummer* (*Homarus*) verfügt nach Verlust der Schreitbeine (Pereiopoden) sogar über mehrere verschiedenartige Fortbewegungsmethoden (Kühl). Beim langsamen Schreiten übernehmen die alternierend bewegten Kieferfüsse (Maxillipeden) die Rolle der Schreitbeine; bei schnellerer Lokomotion stösst der Hummer sich mit den Maxillipeden kräftig vom Boden ab und "springt," unterstützt durch das Schwingen der Schwimmbeine (Pleopoden) durch das Wasser. Schliesslich vermag das Tier sich auch noch mit Hilfe des Abdomens vorwärtszustossen.

Es ist sehr wahrscheinlich, dass alle diese Bewegungsarten im normalen Leben der Tiere nicht vorkommen; ob es sich um Bewegungen handelt, die sich allmählich, nach dem Prinzip des "trial and error" herausbilden, oder ob diese Funktionen sofort in Vollkommenheit auftreten, das scheint in den einzelnen Fällen verschieden zu sein.

Interessant ist schliesslich noch die Beobachtung Kühls (1932) dass der Hummer kurz nach der Häutung, wenn die Schreitbeine noch weich sind, die übliche Koordination völlig aufgibt und stattdessen alle Beine zusammen mit den Scheren synchron in elliptischer Bahn herumbewegt. Diese abweichende Koordinationsweise des weichhäutigen Krebses muss nicht unbedingt als Anpassungserscheinung[1] aufgefasst werden; es ist denkbar, dass die Koordinationsfunktion im Zentralnervensystem selbst sich gleichzeitig mit der Häutung periodisch wandelt.

X. ZUSAMMENFASSUNG.

Die Koordination der Lokomotionsbewegung bei den Arthropoden ist nicht starr festgefügt; die Beinordnung pendelt nur um ein gewisses Gleichgewicht herum. Nach Amputation von Beinen ändert sich dieses Ordnungsgleichgewicht in gesetzmässiger Weise. Dabei können Beine die zuvor synchron bewegt wurden, jetzt miteinander alternieren, und umgekehrt. Zur veränderten Koordination der Bewegung tritt vielfach auch eine Änderung der Haltung.

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[1] Im hier verwendeten Sinne.

Aus dem vergleichenden Studium der normalen Koordination und ihrer Abänderung bei Insekten, Arachnoiden, Crustaceen und Myriopoden lassen sich einige einfache Koordinationsregeln ableiten, die allgemeine Gültigkeit für die Mehrzahl der untersuchten Vertreter aus allen vier Arthropodengruppen besitzen. Diese Regeln besagen, dass bestimmte quantitative Beziehungen zwischen der Anzahl der jeweils vorhandenen Beine und ihrer gegenseitigen Koordination (ihrem Phasenabstand) bestehen. Bei schwimmenden Formen mit besonderer Koordinationsweise bedürfen diese Regeln einer entsprechenden Abänderung.

Die Analyse der einzelnen für die Koordination wichtigen Faktoren ergibt, dass sowohl für die Einordnung jedes Beines in die Gesamtordnung wie für die Form dieser Ordnung selbst in erster Linie die Erregung von Propriozeptoren in jedem Beine während des Laufens massgebend sein muss. Die Koordination richtet sich jeweils nach den im Körper selbst gelegenen Bedingungen ("endoplastischer" Koordinationstyp). Im Gegensatz dazu wird bei einer gewissen Gruppe von Myriopoden die jeweilige Koordination ausschliesslich von den wechselnden Unebenheiten des Laufuntergrundes bestimmt ("exoplastischer" Koordinationstyp).

Es werden die physiologischen Vorstellungen diskutiert, die geeignet sind, ein Verständnis des Koordinationsproblems anzubahnen.

Die Bedeutung des Ober- und Unterschlundganglions für die Koordination der Beine, und der Einfluss, den diese Ganglien überhaupt auf Lokomotion und Koordination des Körpers nehmen können, wird besprochen. Die bei einseitiger Ausschaltung des Hirns auftretende tonische und motorische Asymmetrie des Körpers ist durch elektrische Reizung der durchtrennten Schlundkommissur quantitativ aufhebbar. Diese Asymmetrie bei einseitiger Enthirnung nimmt umgekehrt umso mehr zu, je mehr das Bauchmark von hinten her verkürzt wird.

Zum Schluss werden Beispiele dafür angeführt, dass nach Verlust der Lokomotionsorgane oft ganz neuartige Fortbewegungsweisen zutage treten können.

XI. SUMMARY.

The co-ordination of locomotory movements in arthropods is not completely stereotyped, but the order in which the legs move is tending to a determined equilibrium. When limbs are amputated, the order in which the remaining limbs move becomes altered in a regular manner. Legs which previously moved synchronously may then alternate, and *vice versa*. A change in attitude frequently goes hand in hand with the altered locomotory co-ordination.

Simple rules can be deduced from the comparative study of normal and of artificially changed co-ordination in insects, arachnids, crustaceans and myriapods. These rules apply to the majority of cases investigated in the four classes of arthropods. The rules relate to quantitative relations between the number of legs present and their reciprocal co-ordination (phase differences). In swimming forms, with their special type of co-ordination, the rules are correspondingly modified.

Analysis of the separate factors concerned in co-ordination shows that the stimulation of proprioceptors in each leg during running is principally responsible both for the seriation of the leg in the order of leg movements and for the nature of this order itself. Sometimes co-ordination depends on the varying conditions in the body ("endoplastic co-ordination").

In contrast to this, in a certain group of myriapods the co-ordination is determined solely by the varying unevenness of the ground ("exoplastic co-ordination").

Physiological conceptions are discussed which may lead to an understanding of the problem of co-ordination.

The importance of the supra- and infra-oesophageal ganglia in leg co-ordination is stressed, together with the influence in general of these ganglia on locomotion and co-ordination in the body. Tonic and motorial asymmetry of the body, caused by putting one side of the brain out of action, can be quantitatively eliminated by electrical stimulation of the severed commissure. On the other hand, such asymmetry due to the absence of one side of the brain increases proportionately as the ventral nerve cord is shortened from behind forwards.

In conclusion, examples are given of new types of locomotion which appear after the loss of the normal locomotory organs.

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THE CHEMISTRY OF PEPSIN AND TRYPSIN[1]

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ONE of the most striking peculiarities of living things is the rapidity and precision with which the chemical changes necessary for their existence are carried on. The process of digestion is a familiar example. Proteins are split in the stomach into much smaller compounds, and this process is continued in the small intestines. The final products are precisely those needed for the nutrition of the animal and are formed from proteins with little or no evolution of heat or expenditure of energy. The process cannot be duplicated in the laboratory, since chemical hydrolysis of proteins yields different products and in any case can be accomplished only by violent treatment and the expenditure of considerable energy. Similar examples of the efficiency of the reactions which take place in the animal could be multiplied indefinitely. It is now known that these specific accelerating effects which living cells exert on the reactions occurring within them and in their vicinity are due to the presence of minute amounts of some substances formed by the living cell and which have come to be known as enzymes. Without them life could not exist and yet they themselves are not living.

For many years this property of living matter was regarded as a process of vital activity entirely outside the realm of experimental science. Evidence gradually accumulated, however, to show that the living cell was not necessary for some, at least, of these characteristic reactions; and one case after another was found in which the reaction could be made to take place without the living cell. But it was not until Buchner (1897) discovered that fermentation of sugar could be caused by yeast extract containing no living cells that it was generally admitted that the enzyme was essential rather than the cell itself.

It had been suspected long before Buchner that the process of gastric digestion was due to the presence of some characteristic substance, and Schwann (1836) definitely assumed the existence of such a substance and gave it the name of "pepsin." The existence of trypsin had also been suspected early in the nineteenth century, but was not definitely assumed to exist until the time of Corvisart (1857–8) and of Kühne (1867), who gave it its present name. A large number of other enzymes

were then discovered by means of their characteristic reactions. It was assumed that since these reactions occurred an enzyme must exist to cause them, but there was no direct proof of the actual existence of enzymes, and, in fact, their existence as ordinary chemical compounds has been frequently questioned. The problem was analogous to that of the causative agent of an infectious disease. An agent is assumed to exist because the disease occurs, but the assumption cannot be proved until the etiological factor is actually isolated.

In the meantime the chemists had found that many purely chemical reactions were accelerated by the presence of small amounts of substances which apparently took no actual part in the reaction, and Berzelius (1837) pointed out that the properties of these substances were strikingly similar to those of the active agents found in living cells. He named the general phenomenon catalysis and considered enzymes as a special class of catalysts.

The name "enzyme" was proposed by Kühne (1867) for these organic catalysts. In the last 50 years enzymes and enzymatic reactions have been studied intensively by chemists and physiologists. The chemists have been interested primarily in the mechanism of the reaction and the physiologists in the nature of the reactions, and both chemists and physiologists have spent a great deal of time trying to isolate the enzymes themselves. Rapid progress was made in the study of the nature of the reactions caused by enzymes, but the mechanism by which they caused these reactions to take place and the nature of the enzymes themselves remained quite unknown.

Before discussing what enzymes are, it is well to review what they do. Pepsin and trypsin are typical enzymes, and the reactions which they accelerate are good examples of enzyme reactions in general. Both pepsin and trypsin cause proteins to decompose into smaller molecules but do not carry this process so far as the amino acids which are the ultimate building stones of the proteins. Along with these chemical changes there are marked changes in the physical properties of the protein. If the protein is originally insoluble it is dissolved rapidly by the action of enzymes, and if it is already soluble the viscosity of the solution decreases very markedly. It has often been assumed that, especially in the case of pepsin, these physical changes were not accompanied by any chemical change, but the apparent change in physical properties without accompanying chemical change is simply due, in the writer's opinion, to the fact that the chemical changes are very slight and hard to measure (Northrop, 1929).

According to the current theory of catalytic reactions in general, all the reactions which are observed to take place in the presence of pepsin and trypsin are already occurring, although at an extremely slow rate. The characteristic effects of the enzymes are due to the fact that certain of the very large number of spontaneous reactions are greatly accelerated, while others are not. This property of accelerating certain reactions and not others is referred to as the specificity of the enzymes and is frequently considered to be another of their peculiar characteristics. In reality, however, all chemical reactions are specific and enzyme reactions do not differ qualitatively in this respect from other chemical reactions (cf. Falk, 1924). The

time required for these reactions to occur and the effect of varying the quantity of protein or the quantity of enzyme also differ more or less from the results obtained from simpler chemical reactions, but again the difference is quantitative rather than qualitative, and the anomalous results can usually be shown to be due to some complicated side reaction (Northrop, 1920, 1922 *a*, 1924, 1932 *a*).

Another peculiarity of the action of these enzymes is the fact that pepsin digestion occurs much more rapidly in acid solution than in alkaline solution, while trypsin digestion occurs much more rapidly in alkaline solution than in acid solution. Proteins, when dissolved in acid, are present in the form of acid salts, and when dissolved in alkali are present in the form of alkali salts, and it is probable that trypsin acts only on the alkali salts of the proteins, while pepsin acts only on the acid salts (Northrop, 1922 *b*). There is, apparently, a third class of proteolytic enzymes, like pepsin, which reacts more rapidly with the neutral protein molecule (Willstätter, Grassman and Ambros, 1926). Trypsin differs from pepsin in another respect in that it attacks denatured proteins, *i.e.* proteins which have been heated, very much more rapidly than the native protein. Both enzymes possess the striking property of destroying dead cells rapidly but are not injurious to living cells. The puzzling fact that the stomach and small intestine, although composed largely of protein, are not digested, even though very rapid digestion takes place in the solution with which they are in contact, is an example of this peculiarity. A partial explanation of this difference between living and dead cells was found (Northrop, 1926) to be due to the fact that neither pepsin nor trypsin can enter living cells, whereas they are very rapidly absorbed by dead tissue. If living fish or worms or frogs or bacteria are placed in strong solutions of either pepsin or trypsin, nothing whatever occurs. The organisms are uninjured and live as though they were in a solution of any ordinary protein. Any dead tissue may be dissolved, but the living cells are not injured. In the meantime none of the enzyme is taken up by the tissue of the animal, since the amount of enzyme in the solution remains perfectly constant.

If dead animal tissues are placed in the same solution, they are very rapidly digested. Measurement of the amount of enzyme in the surrounding solution before digestion occurs shows that the enzyme is rapidly taken up by the dead tissue and disappears from the surrounding solution. When the tissue has been digested or dissolved, the enzyme is liberated again. This fact, of course, simply removes one puzzle and substitutes another, since it is now necessary to know why the enzyme should penetrate dead tissue but not living tissue. This puzzle, however, has the advantage of being a very general one and is not at all restricted to enzymes, since, in general, living cells are permeable only to very few substances, while dead cells are easily permeable to almost any substance in solution.

There remains, also, the difficulty of explaining why the enzymes do not digest the surface of the cells, even though they cannot enter. There is good reason to believe that the surface film of cells is not protein, and its behaviour in fact is much more similar to that of an oil, so that this oil-like film is probably the mechanism which prevents living cells from being digested. When the cell dies this film is destroyed and the enzyme enters and digests the protein.

Inhibition of trypsin and pepsin digestion. It was mentioned, in discussing the peculiarities of pepsin digestion, that the course of the reaction was not what would be expected from ordinary chemical theory. It has been found that the quantity of protein digested per minute decreases rapidly as the reaction proceeds. This peculiarity is caused by the inhibitory effect of products formed during digestion on the activity of the enzyme. It may be strikingly demonstrated by adding increasing quantities of these products to the protein solution before the addition of the enzyme (Northrop, 1922 c). The more digestion products are added the slower the digestion; and in the presence of a large amount of digestion products practically no digestion occurs. The enzyme-protein system in some respects closely resembles the toxin-animal system, since the enzyme causes the formation of substances which protect the protein from the effect of the enzyme, just as the injection of toxin into an animal results in the production of antitoxin, which in turn protects the animal from the toxin. The enzyme inhibitor, however, is not nearly so powerful as some antitoxins nor is it protein.

Isolation of crystalline pepsin (Northrop, 1930 a). While the behaviour of enzymes has been systematically worked out in the last 40 or 50 years, very little advance has been made in the knowledge of their chemical nature, so that it has frequently been assumed that they represent an unknown class of compounds. Indirect evidence has been obtained, however, that some, at any rate, are proteins. The rate at which they are destroyed by heat, for instance, is characteristic for the effect of temperature on proteins. The fact that they are adsorbed on finely divided particles is also a property of proteins more than of many other classes of compounds. Pepsin, in particular, seems to have protein-like characteristics, and in fact Pekelharing (1896) isolated an amorphous protein from gastric juice which was highly active and which he considered to be pepsin itself. He was unable, however, to show that the material was a pure substance, and the view that this protein was really the enzyme was never accepted. The writer has repeated Pekelharing's experiments several times in the last 15 years, but until recently had never been able to carry the purification any further. In the meantime Sumner (1926) reported the isolation of a crystalline protein from beans which appears to be the enzyme urease.

Nearly all attempts to isolate enzymes have been done with relatively small quantities of material and in rather dilute solution. Absorption methods have also been extensively used. If enzymes really are proteins, these are not favourable conditions for their isolation, since proteins are extremely unstable in dilute solution and are easily injured by adsorption on surfaces. The attempt to isolate pepsin was again undertaken from the point of view of protein chemistry, using only those conditions under which proteins are relatively stable, *i.e.* concentrated solutions and low temperature. The method was based originally on that of Pekelharing. The last step in Pekelharing's preparation consisted in dialysing a protein fraction from gastric juice against dilute acid. Under these conditions a white precipitate is formed which is a protein and which contains most of the activity. This protein sometimes appears in a somewhat granular form and under the microscope looks as though it might be trying to crystallise. Many attempts were made to crystallise
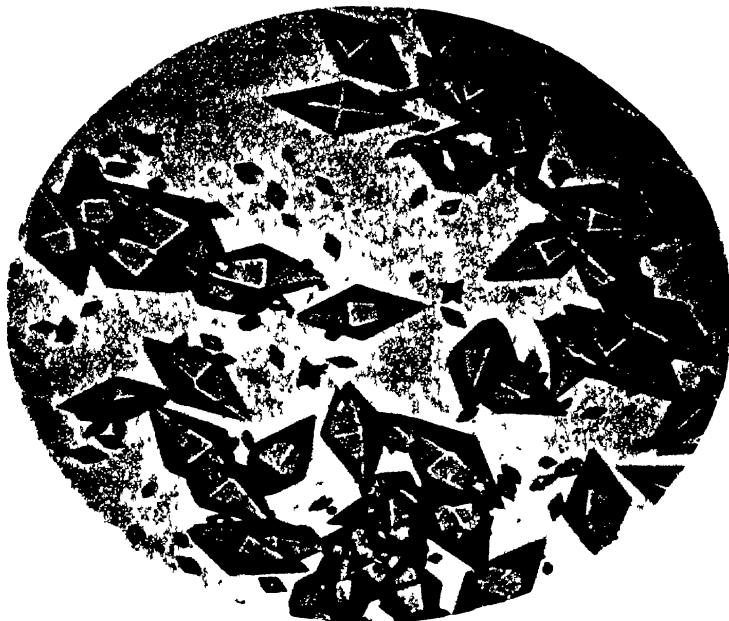
Fig 1 Pepsin
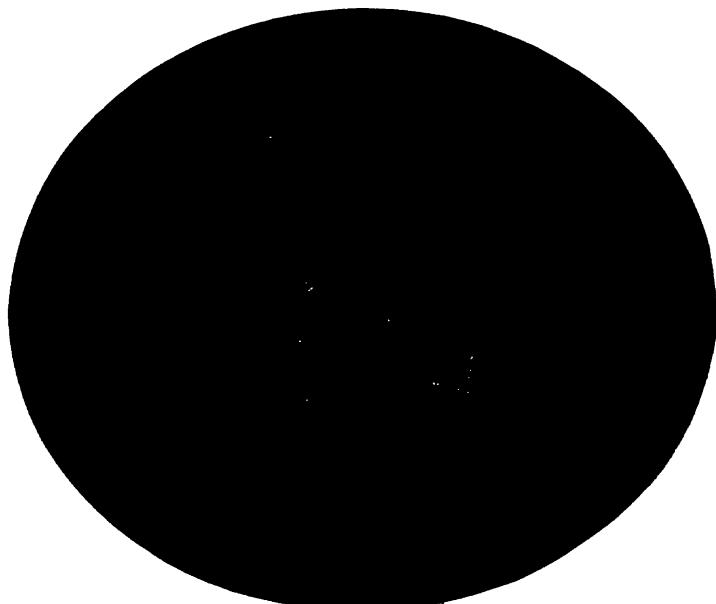
Fig. 2. Trypsin.

the protein without success. It was noticed finally that this precipitate dissolved if the suspension were warmed to 37° C. and reappeared again upon cooling. These are good conditions for the formation of crystals, and the experiment was repeated under varying conditions and especially with more concentrated solutions, since crystallisation in general occurs more readily from concentrated than from dilute solutions. A more concentrated suspension than usual was warmed to 37° C., and this solution was allowed to cool slowly to room temperature in a beaker. The next morning it was found to contain several grams of beautifully formed crystals in the form of double, six-sided pyramids (Fig. 1). They were tested for activity and found to be highly active and also to be protein.

The activity is about five times that of the most highly active commercial preparation, and the quantity of protein which can be transformed by the enzyme is quite extraordinary. A gram of the crystalline pepsin under favourable conditions would digest about 50,000 gm. of boiled egg in 2 hours, or would clot about 100,000 litres of milk, while it would liquefy about 2000 litres of gelatin in the same time. The clotting action on milk is a constant property of the enzyme, but there is probably another enzyme present in gastric extracts which has more powerful milk-clotting activity but much lower proteolytic activity (Tauber and Kleiner, 1933). The prolonged controversy over the question of the identity of pepsin and rennin seems to have been caused by the fact that one enzyme, pepsin, is proteolytic largely but also clots milk, while there is another enzyme, rennin, which clots milk powerfully and has but weak proteolytic activity.

Only small amounts of the crystalline material could be obtained by the original method, but it was found possible to modify it and eventually to dispense with the dialysis which is the most troublesome part of the method. The crystalline protein can now be prepared from commercial pepsin preparations simply by fractionation with magnesium sulphate and then with the proper concentration of sulphuric acid. The protein crystallises very readily, in fact much more readily than most proteins, and it is easily possible to prepare 100 gm. in 2 days. A method was, therefore, at hand by which large quantities of crystalline protein having powerful proteolytic activity could be prepared. The crystalline enzyme may be prepared from gastric juice (Northrop, 1933 a). Pepsin prepared from bovine gastric juice differs from, but is very similar to, that from pig gastric extracts. The two enzymes may be distinguished by solubility measurements. It is probable, therefore, that the various pepsins differ from species to species, as do the haemoglobins.

Isolation of crystalline trypsin (Northrop and Kunitz, 1932 a). An attempt was made to continue the methods used by the earlier workers and to isolate a crystalline protein from pancreatic extracts. The problem turned out to be a difficult one, and a great deal of work was done before any encouraging results in the way of either a crystalline product or a product of constant activity was obtained. The most hopeful method seemed to be a combination of fractionation with acid and salt, as was done in the case of pepsin, but with trypsin it was necessary to use ammonium sulphate. A protein fraction was eventually obtained which had constant activity and gave some indication of crystallisation. The work was made difficult by the very unstable

nature of the protein. This unfortunate property made it impossible to allow a solution to stand for more than a few hours, so that the usual procedure for crystallisation, which consists in allowing a solution to concentrate or cool very slowly, could not be used. After a large number of unsuccessful attempts, Dr Kunitz was able to secure definite, regular crystals by the very cautious addition of strong ammonium sulphate to rather concentrated solutions of the protein. The crystals are rather small and are of the cubic system. The proof that this material is a pure substance is still more difficult than in the case of pepsin, since it is more unstable. A large number of solubility experiments were carried out, but the results were not entirely satisfactory, as it was found impossible to complete the experiments quickly enough to avoid partial decomposition and corresponding loss in activity. The final solutions, therefore, always contained more or less inactive material formed during the progress of the experiments themselves. Several series of solubility measurements were carried out, nevertheless, as rapidly as possible and at 6° C. They were disappointing in that they indicated clearly that the preparation was a mixture. To confirm this result a study was made of the changes in activity when the protein is denatured, as was done with pepsin, except that in this case denaturation was carried out by heating in dilute acid. The trypsin protein when treated in this way becomes denatured and insoluble in the presence of salt. This experiment showed clearly that the preparation, although crystalline, was undoubtedly still a mixture, since a considerable amount of the protein could be coagulated and removed from solution without decreasing the activity of the solution. As the heating was continued, however, and more and more insoluble protein was formed, it was found that the activity began to decrease about in proportion to the formation of insoluble protein. It appeared, therefore, that the original preparation contained two proteins, one of which was easily coagulated by dilute acid and carried no activity with it, while the other one was much more resistant to acid and was associated, at least, with the activity. These results furnished also a further method of purification, since, by heating the crystalline material in dilute acid, about one-third of the protein could be removed without loss in activity. Considerable amounts of the preparation were treated in dilute acid in this way and a second preparation obtained which was about twice as active as the first one. It crystallises more readily than the first preparation and the crystals are similar. The purity of this material was again tested by solubility measurements and the results were more satisfactory than with the first preparation but still not really convincing, owing again to the very unstable nature of the substance. The loss in activity when a solution of this substance was heated in acid was just proportional to the amount of native protein changed to denatured protein.

The crystalline trypsin digests proteins in slightly alkaline solution and has powerful blood-clotting properties. It does not clot milk and differs qualitatively from all previous preparations in that it hydrolyses proteins to a very limited extent. It is thus a purely proteolytic enzyme, and the much greater degree of hydrolysis caused by other preparations is probably due to the presence of other enzymes.

Activation of inactive pancreatic extract by concentrated salt solution (Kunitz and Northrop, 1934*a*). The trypsin described above was isolated from pancreatic extract

which had become active on standing. According to Mellanby and Woolley (1913) this "spontaneous" activation is caused by kinase present in the pancreas. It has recently been found that a material may be obtained from inactive pancreatic extracts which becomes rapidly and completely activated if allowed to stand at 30° C., pH 7·0–8·0, and in half-saturated ammonium or magnesium sulphate. The reaction is autocatalytic and may be accelerated by the addition of previously activated trypsin. Trypsin may thus be "propagated" under these conditions, by inoculating a suspension of inactive pancreatic extract with a little active trypsin.

Isolation of crystalline trypsinogen and its conversion into crystalline trypsin (Kunitz and Northrop, 1934*d*). The inactive extract discussed above contains the inactive
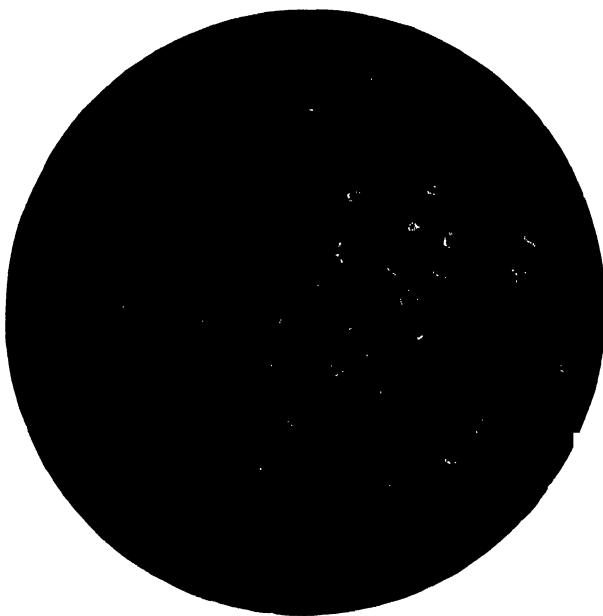
Fig. 3. Trypsinogen.

form of trypsin, trypsinogen. This trypsinogen has been obtained in crystalline form by allowing the solution to stand at 5° C. in half-saturated magnesium sulphate pH 8·0. The crystals are triangular pyramids (Fig. 3). These crystals when dissolved in strong ammonium or magnesium sulphate solutions at pH 8·0 become transformed into the active enzyme, trypsin, which may then be obtained in crystalline form (Fig. 2) under the same conditions as used for crystallising trypsinogen. This is a much more convenient and efficient method of preparing crystalline trypsin than that described above, in which active pancreatic extracts were used as the source of material.

Isolation of crystalline chymo-trypsinogen and chymo-trypsin (Kunitz and Northrop, 1933, 1934*c*). Kühne (1867) and Heidenhain (1874) showed that the proteolytic enzymes of the pancreas are completely inactive in fresh pancreas or in freshly

secreted pancreatic juice. The enzymes become active when mixed with the entero-kinase of the small intestine, as found by Schepowalnikow (1900), or when the pancreas is allowed to stand in slightly acid solution. According to Vernon (1901), activation may also be brought about by small amounts of active trypsin. The mechanism of this activation has been the subject of controversy for many years.

Kunitz and the writer found that an inactive crystalline protein may be obtained from fresh pancreatic extracts. This protein is converted by minute amounts of trypsin into a powerful proteolytic enzyme. This enzyme has also been obtained in crystalline form. The inactive protein was called chymo-trypsinogen (Fig. 4) and the active protein chymo-trypsin (Fig. 5).

Pancreas was removed from cattle immediately after slaughter and immersed in $M/8$ cold sulphuric acid. The pancreas was then minced and extracted for 24 hours at 5° C. with two volumes $M/8$ sulphuric acid. This extract has no measurable proteolytic activity but becomes highly active upon the addition of enterokinase or upon the addition of relatively large amounts of active trypsin. The addition of relatively small amounts of active trypsin does not cause activation. The extract contains a protein which is soluble in 0·4 saturated ammonium sulphate but in-soluble in 0·7 saturated ammonium sulphate. This protein may be crystallised from 0·25 saturated ammonium sulphate by the addition of saturated ammonium sul-phate and adjustment of the pH to about 5·0. It crystallises in the form of elongated prisms. About 1 gm. of crystalline material may be prepared from one beef pancreas. The protein prepared in this way cannot be activated by enterokinase but becomes powerfully active upon the addition of a very small amount of crystalline trypsin or of any crude trypsin solution. The crude extract and the mother liquor from the crystals, on the other hand, are completely activated by kinase but not by small amounts of trypsin. This apparent contradiction is due to the fact that crude extracts and the mother liquor contain some material which inhibits trypsin so that small amounts of trypsin are completely inactivated. When kinase is added to such solu-tions sufficient active trypsin is formed to overcome the inhibiting effect and this active t.ypsin changes the chymo-trypsinogen to chymo-trypsin.

Conversion of chymo-trypsinogen to chymo-trypsin. Three grams of crystalline chymo-trypsinogen were dissolved in 400 ml. $M/30$ pH 7·6 phosphate buffer, 1 mg. of crystalline trypsin added and the solution kept at 5° C. The activity increased rapidly and after 24 hours had reached a constant value of about 1000 times that of the trypsin added. The time rate of increase in activity is logarithmic and not auto-catalytic. This indicates that the chymo-trypsinogen cannot be activated by chymo-trypsin, and control experiments confirm this conclusion. No measurable hydrolysis of the chymo-trypsinogen occurred during activation. The active protein was precipitated from this solution by bringing to 0·7 saturated ammonium sulphate. The filter cake was dissolved in twice its weight of $M/100$ sulphuric acid, ammonium sulphate added to slight turbidity, and the pH adjusted to about 4·0 with sodium hydroxide. The solution was allowed to stand at 22° C. over night and about 2 gm. of a crystalline protein in the form of plates appeared. The activity of this prepara-tion is about one-third that of the previously described crystalline trypsin with
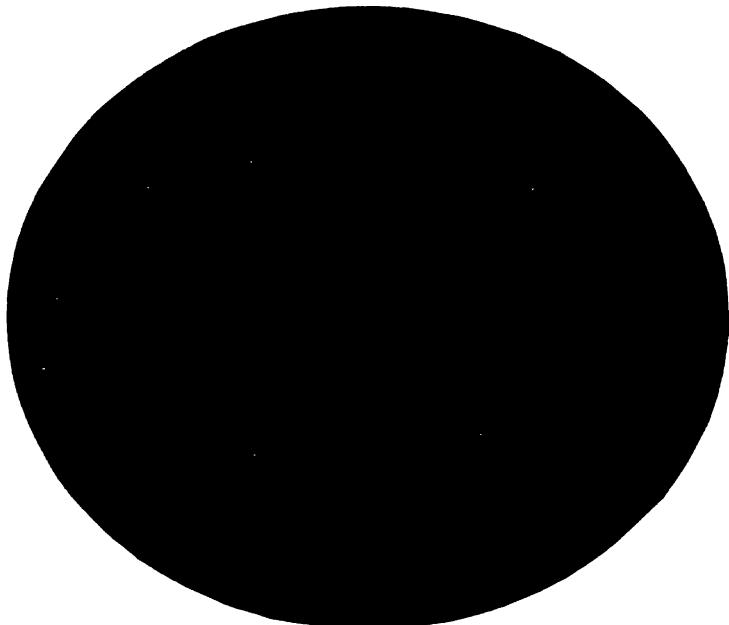
Fig. 4. Chymo-trypsinogen.
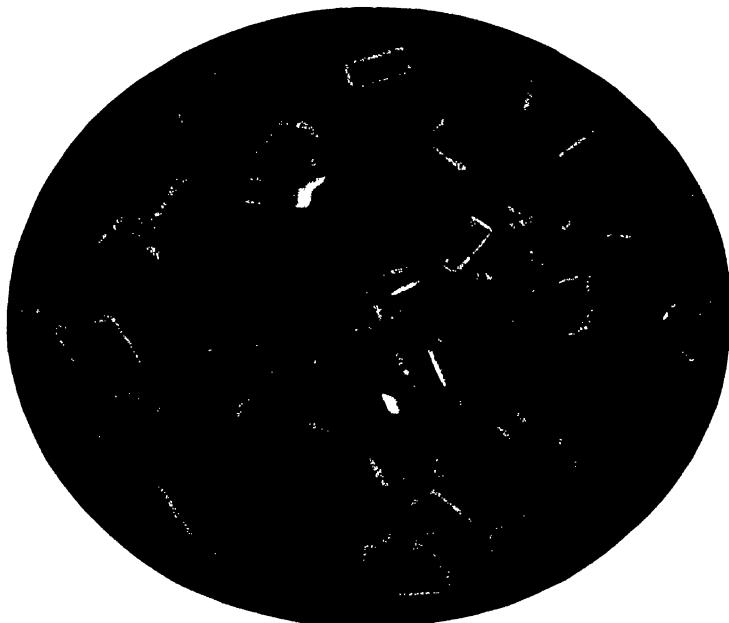
Fig. 5. Chymo-trypsin.

respect to the digestion of haemoglobin or casein. It is much less active than trypsin in liquefaction of gelatin but much more active in clotting milk. It does not clot blood and contains no amylase or lipase activity. The digestion of casein is carried much further than by the crystalline trypsin. The enzyme is evidently quite distinct from the trypsin previously isolated and may represent the "pancreatic rennet" of Vernon.

The chymo-trypsinogen has been recrystallised ten times and shows constant optical activity and constant proteolytic activity after activation by trypsin. Some samples showed a very slight proteolytic activity without activation which was equivalent to about 1/5000 of that of the activated material. This trace of activity is variable and is probably due to the presence of a small amount of active material.

The chymo-trypsin has been recrystallised three times and all fractions show constant optical activity and constant proteolytic activity as measured by digestion of haemoglobin, casein or gelatin, or rennet action.

Evidence that the activity is a property of the protein molecule. The experiments just described have shown that crystalline proteins having intense proteolytic activity may be isolated from gastric or pancreatic extracts. It is an experimental fact that these preparations are crystalline proteins, but it by no means follows directly that the enzymatic activity is a property of the protein molecule. If it could be shown that the preparations are pure substances it would, of course, follow that the activity must be a property of the protein, but it is unfortunately extremely difficult to prove the purity of proteins. Ordinarily a substance is considered to be pure if it can be recrystallised several times without change in properties. Pepsin, trypsin and chymo-trypsin have each been recrystallised from five to ten times under such conditions that a very large percentage of the material is lost during crystallisation; in other words, the preparations have been subjected to fractional crystallisation. No measurable change in physical or chemical properties or in specific activity could be detected after the first one or two recrystallisations. A number of different preparations have been obtained from different lots of raw material and these preparations have also been indistinguishable from one another; the properties of these crystalline enzymes are, therefore, perfectly definite and reproducible. If the compounds were not proteins these results would undoubtedly justify the conclusion that they were pure substances. In the case of proteins, however, it is known, largely from the work of Sörensen (1926), that closely related proteins may be obtained in crystalline form, probably as solid solutions, and that such solid solutions are extremely difficult to separate by fractional crystallisation. Their existence may, however, be detected by solubility measurements. If a pure substance is in equilibrium with a saturated solution the concentration of the material dissolved must be independent of the amount of solid present. In the case of a mixture or a solid solution, however, this is not the case, but the solubility will be found to change with increasing amounts of solid. This is an extremely sensitive test and its use enabled Landsteiner and Heidelberger (1923) to detect the difference between closely related haemoglobins which could not be differentiated by means of specific immune reactions. By means of the same method Sörensen was able to

show that many proteins which had hitherto been considered pure substances were really solid solutions of closely related proteins. Solubility measurements were therefore carried out with crystalline pepsin and it was found that the solubility (Northrop, 1930 a), as determined either by the protein content or the activity of the saturated solution, was independent, within the experimental error, of the amount of solid material present. The experimental error was about ± 10 per cent., and it is quite possible that there is a slight undetected change in solubility with increasing amounts of solid phase. The results are accurate enough, however, to show that no non-protein molecules are present, since it is extremely improbable that a non-protein molecule could be undetected by such measurements. It is possible, however, that the material consists of several very closely related proteins.

The solubility experiments with trypsin (Northrop and Kunitz, 1932 a) are not as conclusive, since the unstable nature of the substance makes it extremely difficult to do accurate solubility measurements. The results show, however, that there was certainly no marked change in solubility with increasing quantities of solid.

The relation of the activity to the protein may be studied in another way by determining the effect of changes in the protein molecule on the activity. If the activity is a property of the protein molecule then it would be expected that any change in activity would be accompanied by a change in the protein molecule, whereas if the activity were due to a non-protein molecule associated with the protein it would be expected that conditions could be found under which the protein could be destroyed without affecting the active molecule. Protein molecules are large and diffuse slowly, so that by measuring the rate of diffusion of the protein directly and also by activity measurements it is possible to show that the rate of diffusion and, hence, the size of the active molecule are the same as those of the protein molecule. It is also possible to determine the molecular weight by such diffusion measurements.

Diffusion experiments have been carried out with pepsin (Northrop, 1930 b), trypsin (Northrop and Kunitz, 1932 b; Scherp, 1933), and chymo-trypsin (Kunitz and Northrop, 1934 c), and in each case it was found that the rate of diffusion, as determined by protein analysis, was exactly equal to that as determined by activity. In other words, the rate of diffusion and, hence, the size of the active molecule were the same as those of the protein molecule.

Pepsin was found to have a molecular weight of about 35,000 by this method, and this figure was confirmed by osmotic pressure measurements and also by measurements with the ultra-centrifuge (Philpot and Eriksson-Quensel, 1933). The ultra-centrifuge method determines only the protein, however, and it is not possible to correlate the activity with the protein by this technique.

Crystalline trypsin was found to have a molecular weight of about 45,000 by the diffusion method and 34,000 by osmotic pressure measurements, showing that the molecule is quite highly hydrated.

The molecular weight of chymo-trypsinogen and of chymo-trypsin was found to be about 52,000 from diffusion measurements and about 40,000 from osmotic pressure measurements, so that these proteins are also hydrated.

Effect of acetylation upon the activity of pepsin (Herriott and Northrop, 1934). Three crystalline acetyl derivatives of pepsin have been obtained by the action of ketene in aqueous solution, pH 4·0–6·0. The first compound which contains 3 or 4 acetyl groups and which has lost all primary amino groups can be isolated after short acetylation (Fig. 6). It has the same activity as the original pepsin. A second derivative containing 6–11 acetyl groups has also been isolated and crystallised (Fig. 7). It has 50–60 per cent. of the activity of the original pepsin. A third derivative having 20–30 acetyl groups and about 10 per cent. of the activity of the original pepsin can be isolated after prolonged acetylation. Fractionation and solubility experiments show that these preparations are not mixtures or solid solutions of the original pepsin with an inactive derivative. The 60 per cent. active derivative on standing in strong acid solution loses most of its acetyl groups and at the same time regains most of the activity of the original pepsin. The compound obtained in this way is probably the same as the completely active acetyl derivative obtained by mild acetylation.

These results show that acetylation of the primary amino groups of pepsin causes no change in the activity of the enzyme, but the introduction of acetyl groups in other parts of the molecule results in a marked loss in activity. These concomitant changes in chemical nature and in activity caused by the introduction or removal of acetyl groups furnish good evidence that the activity is a property of the protein.

Chemical changes accompanying inactivation of the enzymes: (a) Irreversible inactivation. Enzymes in general are inactivated by strong alkali or acid, high temperatures, ultra-violet light and exposure to radium. They may also be inactivated by other enzymes. The loss in activity of pepsin and trypsin under various conditions has been followed in detail, and it has been found that this loss in activity is accompanied by a corresponding loss in native protein so that whenever a certain fraction of the original activity was found to have disappeared the same fraction of the original native protein has also disappeared. Thus, if pepsin solutions were titrated to various pH's between 6·0 and 9·0 and then brought back to pH 4·0 it was found that there was an increasingly great loss in activity as the solutions were made more alkaline (Northrop, 1931). There was at the same time an increasingly large percentage of the original protein changed to the denatured form, so that under these conditions the formation of denatured protein and the loss in activity are parallel. Pepsin solutions in strong acid (Northrop, 1932 b), or when exposed to ultra-violet light or to gamma rays from radium (Northrop, 1934), also lose activity quite rapidly, but in this case no denatured protein appears but merely decomposition products. The protein is probably first changed to the denatured form since in strong acid denatured protein appears (Herriott and Northrop, 1934), and this denatured protein is then hydrolysed by the remaining active protein. Under these various conditions also the loss in activity is just proportional to the loss in native protein. The fact that the final solution has no measurable activity shows that none of the pieces derived from the original protein has any appreciable activity.

The identity of the active and of the protein molecule has been further confirmed by Gates' (1934) experiments with ultra-violet light. The ultra-violet absorp-
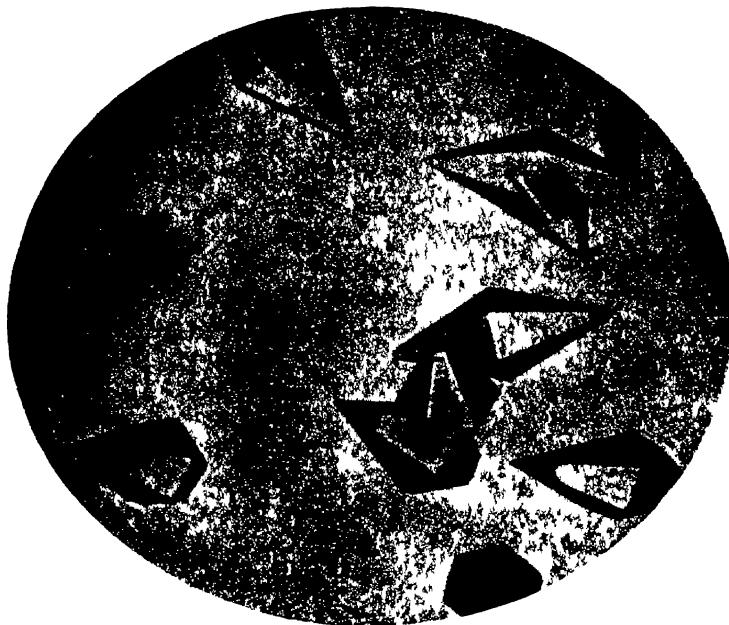
Fig 6　100 per cent active acetyl pepsin
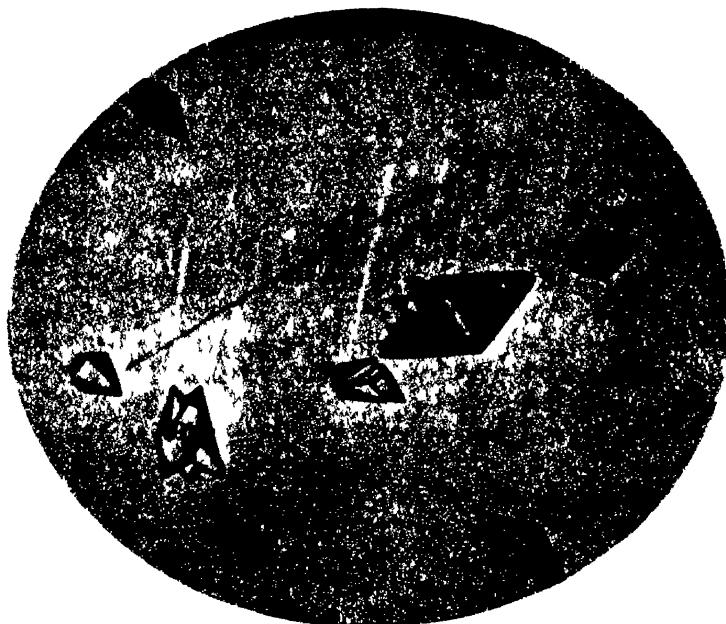
Fig. 7. 60 per cent. active acetyl pepsin

tion spectrum of pepsin solutions may be determined directly by photographic measurements. The extent to which a pepsin solution absorbs the various wave-lengths was determined in this way. This absorption must be due to the protein, since the curve for the absorption spectrum obtained is that of a typical protein, and since the absorption coefficients are extremely high and agree with those usually found for proteins. Inactivation experiments show that the inactivating efficiency of the various wave-lengths may be predicted from their absorption coefficients and, further, that for each quantum of radiant energy absorbed about one molecule of pepsin is inactivated. These experiments show, therefore, that the relative absorption of the various wave-lengths by the active molecule, as measured by their inactivating effect, is the same as their absorption by the protein, as measured photographically. This result is difficult to account for except by assuming that the two molecules are identical. Similar experiments have been carried out by Kubowitz and Haas (1933) with urease. In this case also the inactivation efficiency of the various wave-lengths is that predicted by their absorption coefficients.

The inactivation of trypsin in aqueous solution at temperatures below 37° C. was studied in detail and was found to depend strikingly upon the pH of the solution (Kunitz and Northrop, 1934 b). On the acid side of pH 2·0 the trypsin protein is changed to an inactive protein which differs from the original in that it is irreversibly denatured by heat. The course of this reaction is monomolecular and the velocity increases as the acidity increases. From pH 2·0 to 9·0 the trypsin protein is hydrolysed. The course of the inactivation reaction in this range is bimolecular and its velocity increases as the alkalinity increases to pH 10·0 and then decreases. On the alkaline side of pH 13·0 the reaction is similar to that in strong acid solution and consists in the formation of inactive protein. The course of the reaction is monomolecular and the velocity increases with increasing alkalinity. From pH 9·0 to 12·0 some hydrolysis takes place and some inactive protein is formed and the course of the reaction is represented by the sum of a bi- and monomolecular reaction. In general the decrease in activity under all these conditions is proportional to the decrease in the concentration of trypsin protein. Trypsin in acid solution is hydrolysed by pepsin, and in this case again the loss in activity is just proportional to the amount of trypsin protein hydrolysed.

In the experiments just described the activity was determined by the hydrolysis of proteins in solution. Schulman and Rideal (1933) have found, however, that, if the effect of the enzyme on the surface film of protein is used as a measure of activity, autolysed or digested trypsin solutions still possess the ability to affect the surface film. It is possible that some fragment of the protein molecule is capable of acting upon protein when present as a surface film. If this is true the method furnishes an extremely valuable means for identification of that part of the enzyme molecule responsible for the activity.

The loss in activity of chymo-trypsin solutions during pepsin digestion or by high temperatures or strong acid has also been studied (Kunitz and Northrop, 1934 c). In this case as well the loss in activity is accompanied by a corresponding loss in

native protein. Further evidence of the protein nature of chymo-trypsin may be found in the fact that the activation of chymo-trypsinogen is caused by trypsin. So far as is known trypsin has no action on any class of compounds except proteins, and since it transforms chymo-trypsinogen into chymo-trypsin the probabilities are that the reaction consists of a change in a protein molecule. This conclusion is borne out by the fact that the transformation of chymo-trypsinogen into chymo-trypsin is accompanied by an increase of six primary amino groups per mole.

(b) *Reversible inactivation.* If pepsin solutions are titrated to pH 9·0 or 10·0 the pepsin protein is completely denatured and the solution is inactive. If the solutions are titrated back to pH 5·4 and allowed to stand for several hours the activity gradually returns and at the same time a proportionate amount of native protein is reformed from the denatured protein (Northrop, 1931; Swaetichin, 1932). In concentrated solutions the yield is very small but becomes greater as the solutions are made more dilute. These results show that the activity is lost when the native protein is changed to the denatured form and is recovered when the denaturation reaction is reversed.

Trypsin in acid solution and low temperatures is quite stable and exists entirely as native protein. As the temperature is raised, however, the native protein is transformed to denatured protein and the activity is lost, so that at 60° C. the protein is all in the denatured form and the solution is inactive (Northrop, 1932 c; Anson and Mirsky, 1934). This reaction is rapidly and completely reversible, and as the solution cools the activity returns and the protein is again in the native state. This equilibrium follows quite accurately Van 't Hoff's equation between heat of reaction and the effect of temperature with the value of 67,600 calories per mole.

A similar reversible formation of denatured protein occurs in trypsin solutions at lower temperatures when the pH of the solution is greater than 7·0 (Kunitz and Northrop, 1934 b). The percentage of trypsin present in the denatured form increases rapidly with increasing alkalinity until at pH 13·0 practically all of the trypsin is inactive and denatured. This reaction is reversible only for a very short time in solutions of purified trypsin. Under these conditions also the loss in activity is proportional to the amount of denatured protein formed. This result is direct experimental evidence confirming the hypothesis of Michaelis and Davidsohn (1911) that the effect of pH upon the activity of trypsin is due to an equilibrium between the active and inactive form of the protein. The writer found (Northrop, 1922 b) that the pH optimum for trypsin digestion varies with different proteins and suggested that trypsin reacts with negative protein ions, but it is difficult on this basis to account for the subsequent decrease in the rate of digestion as the solution becomes still more alkaline. The effect of pH upon the ionisation of the protein together with the present experiments furnishes a complete picture of the pH activity curve of trypsin.

Adsorption of pepsin by foreign proteins. If the pepsin protein acted merely as a carrier for some active non-protein molecule it might be expected that other proteins could be substituted for the pepsin protein and in this way a series of active proteins obtained. As a matter of fact it has long been known (Dauwe, 1905;

Northrop, 1919; Dyckerhoff and Tewes, 1933) that inert proteins suspended in solutions of pepsin removed most of the activity, and Waldschmidt-Leitz and Kofrányi (1933) have assumed that this reaction was due to removal of an active group from the pepsin protein and its attachment to the foreign protein. A detailed study of the reaction (Sumner, 1933; Northrop, 1933 *b*), however, shows that the pepsin protein itself is taken up by the foreign protein so that a complex of pepsin-foreign protein is obtained. This complex may contain surprisingly large amounts of pepsin protein and is correspondingly active. Edestin crystals may be made to take up 20 per cent. of their own weight of pepsin without change in their physical appearance. The pepsin may be removed and recovered from this complex by washing in strong acid or by allowing the complex to autolyse. In this case the edestin is hydrolysed and the pepsin remains and may be recrystallised.

Discussion. The experiments described in this article show that crystalline proteins may be isolated which have constant physical and chemical properties including intense proteolytic activity. The proteins have been studied under a variety of conditions which would be expected to show evidence of mixtures without causing any demonstrable change in their characteristic properties. If the materials were other than proteins these experiments would justify the statement that they were pure substances. Since they are proteins, however, it is quite possible that the material may be a solid solution, as in the case of proteins such solid solutions frequently exist and are extremely difficult to fractionate into their components. The problem is rendered unusually difficult in this case by the extremely unstable nature of the proteins. It seems unlikely, however, that the materials contain any non-protein molecular species. The constant composition under various conditions of fractionation precludes the possibility of an adsorption compound, since it is characteristic of these compounds that their composition varies with the conditions of precipitation.

Even though the crystalline materials are a mixture or solid solution and not pure substances, there seems good reason to believe that the proteolytic activity and protein properties are attributes of the same molecule. This conclusion is confirmed by a number of experiments in which it was found that any change in the protein properties caused a corresponding decrease in the activity of the solution. Denaturation of the proteins by heat, hydrolysis by acid or alkali all cause the concentration of native protein in the solution to decrease, and this decrease is accompanied by a corresponding decrease in activity. In addition, the denatured, inactive protein formed by heating the solution reverts to the native condition when the solution is cooled and at the same time the normal specific activity returns. In order to account for these results on the assumption that the activity is due to the presence of some non-protein molecule, it is necessary to assume that this hypothetical molecule becomes inactive when the protein is denatured, and also that it regains its activity at the same time and under the same conditions as cause the denatured protein to return to the native form. In the absence of positive proof for the existence of such a hypothetical molecule these assumptions seem unlikely. So far as the writer is aware, there is no positive proof of the existence of such molecules, and the assump-

tion that they exist rests merely on the negative fact that most of the attempts to prepare pure substances, *i.e.* those with constant properties including enzymatic activity, have been unsuccessful. On the other hand it is, of course, impossible to disprove the existence of such molecules. Since nothing is known of the properties of these hypothetical active molecules it would be perfectly logical to assume that they are proteins themselves, especially since the general properties of enzymes such as inactivation by heat, adsorption on surfaces, and destruction by strong acid or alkali are in general those of proteins.

Active enzyme preparations have been obtained which contain very small amounts of protein; on the other hand extremely active preparations of urease, pepsin and trypsin and amylase have been obtained which are pure, or nearly pure proteins. If it be assumed that the activity of these protein preparations is due to the presence of some minute amount of a non-protein molecule, it is equally reasonable to assume that the activity of the non-protein preparation is due to the presence of a minute amount of protein.

The fact that in other cases the enzymatic activity may vary independently of the total protein content of the preparation proves only that some of the protein present is inactive but not that all of it is inactive.

Numerous experiments have been reported in the literature in which solutions of pepsin and other enzymes have been found to give negative protein tests although they are active. These experiments are also inconclusive, since the activity test is far more delicate than the chemical test for proteins. For instance, solutions of crystalline trypsin or pepsin containing less than $1/1,000,000$ of a gram of protein nitrogen per ml. have an accurately measurable effect on the digestion of casein, while solutions of pepsin containing less than $1/10,000,000$ of a gram of nitrogen per ml. have a very powerful effect on the coagulation of milk. Such solutions give negative results with protein tests, but the dry material from which the solutions are made is practically pure protein. The minimum concentration of these enzymes which can be detected is at least ten times less than the concentration mentioned above and is of the same order of magnitude as the concentration of respiratory ferment in yeast as calculated by Warburg and Kubowitz (1928).

It appears to the writer, therefore, that the assumption that enzymes are proteins is in the best accord with the facts up to the present time. Since these proteins possess characteristic enzymatic activity, in addition to the usual properties of proteins, they must possess some characteristic chemical structures which may or may not be an amino acid complex. The problem is the same as in the case of insulin. In general, most properties of molecules cannot be considered quantitatively as the sum of the properties of the various groups or atoms of which they are made, but must be considered as properties of the whole molecule. Thus, the optical activity, colour, strength of acid groups, etc., of any one molecule depends qualitatively on the presence of a certain group or groups, but quantitatively the property is affected by any change in the molecule. For instance, most optically active molecules contain an asymmetric atom, but the specific optical activity will change with any change in the molecule and it is impossible to isolate a group from the compound

possessing the optical activity of the whole molecule. The same is true of the colour of dyes to a more marked degree.

Haemoglobin presents perhaps the best analogy. This substance has the general properties of a protein, but in addition possesses the remarkable property of combining reversibly with oxygen. It acts as a catalyst in certain oxidation reactions and might, therefore, be considered an enzyme. The property of combining reversibly with oxygen is assumed to be due to the presence of the iron-pyrrol (prosthetic) group, but denaturation of the haemoglobin, a reaction common to all proteins, destroys its power of combining with oxygen although the denatured protein still contains the prosthetic group.

Krebs (1928) has shown that haem itself is a very poor catalyst but when combined with certain nitrogenous groups it forms haemochromogens, some of which are very effective catalysts. Thus the catalytic properties of haemoglobin and these related compounds are all due to the presence of the haem group, but this group when isolated has little or no catalytic activity, and the catalytic power of the various haem compounds depends upon the substance with which the haem is combined. It is quite possible that the same general condition applies to other enzymes and that there are an indefinite number of closely related enzymes depending upon the compound with which the characteristic group is combined. This point of view does not differ very much from that developed by Willstätter (1922) and his collaborators (Waldschmidt-Leitz, 1933), except that it regards the various active compounds as definite chemical individuals rather than as adsorption complexes of varying composition.

Theorell (1934) has recently obtained the yellow respiratory enzyme of Warburg in crystalline form. The enzyme is a protein and, like haemoglobin, contains a prosthetic coloured group. The enzyme may be separated into a simple protein and this prosthetic group, but neither part alone has any activity. Thus the respiratory ferment is quite similar to haemoglobin in general chemical configuration.

At the present time, however, there is no direct evidence of the existence of any peculiar prosthetic group in the other crystalline enzymes not found in other proteins, and it is quite possible that their activity depends on some peculiar arrangement of the amino acids, as Jensen and Evans (1934) have suggested in the case of insulin.

Sörensen (1926) has shown that protein solutions in the presence of the solid phase are in true equilibrium and that the system as a whole is a two-phase one as defined by the phase rule. The protein solution, therefore, consists of one phase. The solubility experiments with pepsin and trypsin give the same result. These results show that the catalytic reactions caused by pepsin and trypsin in protein solutions are homogeneous rather than heterogeneous.

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DIE PHYSIOLOGIE DES FACETTENAUGES

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INHALT.

IM folgenden soll versucht werden, eine kurze Übersicht zu geben über unsere derzeitigen Kenntnisse von der Funktion und Leistung des Facettenauges. Die letzte derartige Zusammenfassung wurde von Demoll (1917) geschrieben. Aus seiner Darstellung ergibt sich, dass um diese Zeit erst verhältnismässig wenig mit dem lebendigen Facettenauge experimentiert worden war, sehr vieles, was man von der Physicłogie dieser Augen zu sagen wusste, bezog sich auf das, was man vom Bau des Auges auf seine Funktion schliessen kann. Seit dieser Zeit ist eine grundlegende Wandlung eingetreten. Zahlreiche Experimentatoren haben sich, ausgehend von sehr verschiedenen Fragestellungen, mit dem Facettenauge beschäftigt, und unsere Kenntnisse sind rasch im Wachsen. Es ist daher vielleicht gerechtfertigt, einen Augenblick auf diesem Wege stehen zu bleiben und, rückwärts schauend, den durchmessenen Weg zu überblicken. Es soll hier nur von unserem experimentalem Wissen die Rede sein. Der anatomische Bau des Facettenauges und die aus ihm unmittelbar abzuleitenden Schlüsse auf die Funktion sollen ausserhalb der Erörterung bleiben.

I. DIE LEISTUNGEN DES EINZELNEN OMMATIDIUMS.

Immerhin müssen wir auf die anatomische Grundtatsache zurückgreifen, dass das Facettenauge sich zusammensetzt aus einer meist grösseren Zahl von Einzelaugen oder Ommatidien. Hieraus ergibt sich sofort eine Disposition. Wir können

erstens fragen, was leistet dieses Einzelauge für sich betrachtet, und zweitens, was leistet die Gesamtheit dieser Einzelaugen, die sich zu dem, was wir Facettenauge nennen, zusammenschliessen.

(1) Das Ommatidium als physiologische Einheit.

Die Anatomen sehen in dem Ommatidium eine geschlossene Einheit; die Schulmeinung geht dahin, dass es in jedem Falle nur einen einzigen unteilbaren Eindruck dem Gehirn weiter leiten kann und dementsprechend genau so wirkt wie ein einzelnes Sehelement (Stäbchen oder Zapfen) der Wirbeltierretina. Das Ommatidium kann nach dieser Auffassung nur eine Änderung des einfallenden Lichtes percipieren, weiter nichts.

Allerdings gilt diese Hypothese nur mit der folgenden Einschränkung. Der spanische Forscher Cajal (1915) wies schon nach, dass in der Retina der Insekten zwei verschiedene Sorten von Sehzellen vorkommen: kurze, die nur bis zum äussersten Teile des Lobus opticus, der sogenannten Lamina ganglionaris, reichen und hier zu mehreren mit einer Ganglienzelle in Verbindung treten, und lange, die sich bis zur Medulla externa verfolgen lassen und meist einzeln an ein Neuron herantreten. Gewöhnlich wird angenommen, dass die langen Zellen unseren Zapfen entsprechen und das Farbensehen vermitteln. Für die anderen stäbchenartigen kann im oben angegebenen Sinne die Einheitshypothese aufrecht erhalten werden.

Physiologische Beweise dafür, dass das Ommatidium wirklich die Seheinheit des Facettenauges ist, gibt es zur Zeit erst wenige. Buddenbrock und Schulz (1933) konnten für eine Reihe verschiedener Insekten sowie für Asseln den Nachweis führen, dass bei der Lichtkompassreaktion (vgl. S. 303) eine Reaktion des Tieres nur dann eintritt, wenn der Lichtstrahl von einem Ommatidium ins nächste übertritt. Bleibt der leuchtende Punkt, auf den sich das Tier eingestellt hat, bei seiner Verschiebung im Sehraum des Ommatidiums, so tritt keine Reaktion ein. Interessant ist hierbei, dass auch für sehr primitive Facettenaugen mit wenigen, weit geöffneten Ommen (*Forficula, Oniscus*) die gleiche Beziehung gilt.

Als ein zweiter Beweis für die physiologische Einheitlichkeit des Ommatidiums können die Beobachtungen gelten, die Keffer und Graham (1932) bei der Messung der Aktionsströme des Limulusauges machten. Es ergab sich, dass man eine ziemlich komplicierte Kurve erhält, wenn man den Aktionsstrom vom gesamten Augennerven abnimmt, dagegen ein sehr einfaches Bild, wenn man den Strom von einem Nervenbündel abnimmt, das von einem isoliert belichteten Ommatidium ausgeht. In diesem Falle (Fig. 1, A, B) ergibt weitere Aufspaltung des Nervenbündels keine noch einfachere Erregungsform, sodass wir berechtigt sind zu sagen, dass jedes Ommatidium eine einheitliche Erregung zum Gehirn schickt.

Weniger zufriedenstellend sind die Resultate, die man bei der sogenannten Sehschärfenprüfung der Insekten erlangt hat. Wenn man am Auge eines Insekts oder Krebses ein optisch differenziertes Muster, z. B. ein System paralleler schwarzer und weisser Streifen vorbeibewegt, so macht das Tier entsprechende Bewegungen (vgl. S. 292). Indem man das Streifenmuster allmählich verfeinert, gelangt man schliesslich zu einer Grenze, über die hinaus eine Reaktion nicht mehr

zu erlangen ist. Hecht und Wolf (1929) fanden nun, dass bei der Biene diese Grenze erreicht ist, wenn der einzelne Streifen unter einem Winkel von 1 Grad gesehen wird. Da nun Baumgärtner (1928) gefunden hatte, dass dieser Winkel der geringste Ommatidienwinkel ist, so schlossen sie aus der Übereinstimmung zwischen dem Ommenwinkel und dem minimalen Sehwinkel, der in ihren Versuchen noch eine Reaktion ergab, dass sie experimentell das Auflösungsvermögen des Bienenauges festgestellt hätten und das Ommatidium die physiologische Seheinheit sei. Indessen stehen dieser Beweisführung erhebliche Bedenken entgegen. Vor allem hat Hertz (1934) überzeugend nachgewiesen, dass bei einer Streifenbreite, die mit dem Öffnungswinkel identisch ist, das Tier nur noch ein Flimmern, aber keine gerichtete Bewegung mehr erkennen kann. Anderseits zeigte die gleiche Autorin (1934) dass unter Umständen noch sehr viel feinere Muster, die an dem Tier vorbeibewegt werden, noch als bewegt erkannt werden können. Es ergibt sich hieraus, dass man auf diesem Wege kaum einen exakten Beweis für die physiologische Einheitlichkeit des Ommatidiums erbringen kann.

Fig. 1. Aktionsströme vom Seitenauge von *Limulus*. A und B, Aktionsströme von je einem Ommatidium; C, von beiden zusammen; D, von drei Ommatidien. (Nach Keffer und Graham, 1932.)

Es hat nichts mit der Frage nach der physiologischen Seheinheit zu tun, wenn wir bei anderen Experimenten die Erfahrung machen, dass das Tier nur anspricht, wenn eine gewisse Anzahl von Ommatidien zugleich gereizt werden. Wir wissen dann eben nur, dass Reizung einer kleineren Ommatidienzahl zur Auslösung einer Bewegungsreaktion nicht langt. So reagiert der Einsiedlerkrebs *Eupaourus* auf sich bewegende Gegenstände mit einem Schlage seiner zweiten Antennen (vgl. S. 291). Bedingung für diese Signalreaktion ist aber, dass ihm der Gegenstand unter einem Winkel von *ca.* 5·4–6·2 Grad erscheint (Bröker, 1934). Es müssen also mindestens etwa 7 Ommatidien gereizt werden. Baumgärtner (1928) stellte fest, dass die Biene auf ein Quadrat von 10–11 mm. Kantenlänge erst aus einer Entfernung von 10–11 cm. anspricht. Hierbei werden linear etwa 5–6 Ommatidien gereizt, insgesamt *ca.* 25. Besonders gross ist die erforderliche Ommenzahl, wenn eine Fluchtreaktion ausgelöst werden soll. Eine gleichzeitige Reizung vieler Ommatidien ist auch Bedingung für die sogenannte optomotorische Reaktion (vgl. S. 292).

(2) Das Unterscheidungsvermögen.

Die Frage, welche geringste Helligkeiten das Facettenauge zu unterscheiden vermag, kann zwar nur an den Reaktionen des ganzen Tieres studiert werden, das Gefundene bezieht sich aber notwendiger-weise auf das einzelne Ommatidium und ist daher in diesem Kapitel zu erörtern. Bisher liegen nur sehr wenige Untersuchungen über diesen Gegenstand vor. Es sind zwei ver-schiedene Methoden entwickelt worden. Wolf (1933) untersuchte die Honigbiene mit einer Variante derjenigen Methode, die von Hecht und ihm bei der Sehschärfeprüfung der Biene verwendet worden war. Unter dem Glasbehälter, in dem das Versuchstier sich befindet, ist eine Opalglasscheibe angebracht, auf deren Unterseite schwarze Papierstreifen geklebt sind. Indem die Scheibe mit auffal-lendem und durchfallendem Licht beleuchtet wird, lässt sich jede beliebige Helligkeits-differenz zwischen den durchsichtigen Glas-teilen und den mit Schwarz unterklebten erzielen. Geprüft wird, auf welche geringsten Differenzen das Tier bei Hin- und Her-bewegen der Glasplatte anspricht. Die Resul-tate zeigt die nebenstehende Figur 2. Man erkennt, dass die Unterschiedsempfindlich-keit sehr von der Lichtstärke abhängt, und dass sie ziemlich gering ist. Wolf schliesst aus seinen Befunden, dass die Biene selbst im günstigsten Falle nur den zwanzigsten Teil des Unterscheidungsvermögens des Menschenauges besitzt.

Fig. 2. Abhängigkeit des Helligkeitsunter-scheidungsvermögens der Biene von der Lichtintensität. (Nach E. Wolf, 1933.)

Buddenbrock (1934) prüfte verschiedene Insekten mit einer anderen Methode. Genau wie bei Wolf werden die optomotorischen Reaktionen zur Prüfung des Tieres benutzt, das sich in einem kleinen Glasgefäss ruhend im Mittelpunkt eines Papiercylinders befindet, der um eine senkrechte Achse rotiert. Auf dem Papier sind senkrechte schwarze Streifen gemalt, die so schmal sind, dass sie der Breite nach bei der gegebenen Entfernung nur einen Bruchteil des Sehraums eines Ommatidiums erfüllen.

Die einzelnen Streifen liegen so weit auseinander, dass zwischen ihnen stets eine grössere Zahl ungereizter Ommatidien sich befindet. Bei dieser sehr einfachen Anordnung zeigt es sich, dass die Insekten noch auf Streifen ansprechen, die sie unter einem ausserordentlich kleinen Winkel sehen (s. Tabelle I). Dementspre-chend ergeben die allerdings nur ungefähren Berechnungen eine Reaktion auf verhältnismässig geringe Helligkeitsdifferenzen. Die wahre Schwelle dürfte noch ein Beträchtliches tiefer liegen, da bei der angewandten Berechnung die Verdunk-

lung durch den vorbeiziehenden Streifen grösser erscheint als sie ist. Die wirkliche "Unterschiedsempfindlichkeit" des Insektenauges lässt sich, wie selbstverständlich ist, überhaupt nicht feststellen. Was aus den Versuchen von Wolf und von Buddenbrock zu erkennen ist, ist lediglich die Schwelle für die geschilderte motorische Reaktion.

Es geht aus diesen vorläufigen Untersuchungen hervor, dass sich die verschiedenen Insektengruppen auch in ihrem Unterscheidungsvermögen sehr erheblich unterscheiden. Das leistungsfähigste Auge scheinen die Fliegen zu besitzen.

Tabelle I.

Lichtstärke	*Coccinella septempunctata*			*Apis mellifica*		
	Minim. Streifenbr.	Sehwinkel	Verdunklung	Minim. Streifenbr.	Sehwinkel	Verdunklung
0·6 lux	5 mm.	3° 12′	96 %	1·5 mm.	57·6′	95 %
13·8 lux	2–3 mm.	1° 36′	60 %	0·6 mm.	22·8′	40 %
200 lux	1·2 mm.	45·6′	24 %	—	—	—

Lichtstärke	*Pieris brassicae*			*Eristalis tenax*		
	Minim. Streifenbr.	Sehwinkel	Verdunklung	Minim. Streifenbr.	Sehwinkel	Verdunklung
0·6 lux	0·6 mm.	22·8′	—	0·35 mm.	13·2′	—
13·8 lux	0·25 mm.	9·6′	—	0·17 mm.	6·5′	—
200 lux	0·17 mm.	6·5′	—	0·1 mm.	3·8′	—

(3) DIE ADAPTATION.

Es ist selbstverständlich, dass das Facettenauge genau so wie die anderen Augentypen über die Fähigkeit verfügt, seine Empfindlichkeit mit der Lichtstärke zu ändern, mit anderen Worten sich zu adaptieren. Die Adaptation wird zwar gewöhnlich am ganzen Tiere studiert, bezieht sich aber selbstverständlich auf jedes einzelne Ommatidium und ist daher ebenfalls hier zu besprechen. Zur quantitativen Untersuchung dieser Verhältnisse sind verschiedene Methoden entwickelt worden. Stehr (1931) benutzte die Tatsache, dass gewisse Wasserinsekten auf plötzliche Belichtung mit einer Schwimmbewegung antworten. Die Reaktionszeit, d. h. die Zeit, die zwischen dem Reiz und dem Beginn der Reaktion liegt, ist hierbei abhängig von der Lichtstärke. Lässt man ein Tier sich an eine bestimmte Lichtstärke adaptieren und bringt es hierauf verschieden lange Zeit ins Dunkel, so reagiert es, wieder in das gleiche Licht zurückgebracht, natürlich auch erst nach einer bestimmten Reaktionszeit. Sie ist umso kürzer, je besser sich das Tier inzwischen an die Dunkelheit adaptiert hat. Man erhält also eine sehr typische Adaptationskurve, die aufs beste mit denen übereinstimmt, die man von den verschiedensten anderen Tieren her kennt. Zu dem selben Ergebnis kam auch H. Keffer (1930), der die Dunkeladaptation von *Limulus* mit Hilfe der Aktionsströme studierte, die sich von

den Augennerven ableiten lassen. Welsh (1930a) studierte am dekapoden *Palae-
monetes vulgaris* die Wanderung der Pigmentzellen (vgl. S. 290), die im Mikroskop
gemessen werden kann. Die Resultate sind, wie Fig. 3 zeigt, im wesentlichen die
gleichen. Die Adaptation geht erst sehr schnell und dann immer langsamer vor sich.

Aus dem Geschilderten ergibt sich, dass das Facettenauge hinsichtlich der
Adaptationsvorgänge in den Sinneszellen völlig mit den anderen Lichtsinnes-
organen übereinstimmt. Es ist aber wahrscheinlich, dass die Adaptationsvorgänge,
auch die der höheren Tiere, sich nicht auf diese Processe in den Receptoren be-
schränken, sondern sich ausserdem in den nervösen Centren abspielen. Hierfür
gibt es auch beim Facettenauge einige Belege.

So konnten Buddenbrock und Schulz (1933) bei der Sehschärfeprüfung ver-
schiedener Käfer nachweisen, dass von einer gewissen Belichtung abwärts die Tiere
sich so verhielten, als seien ihre Seheinheiten linear um das Dreifache gewachsen.
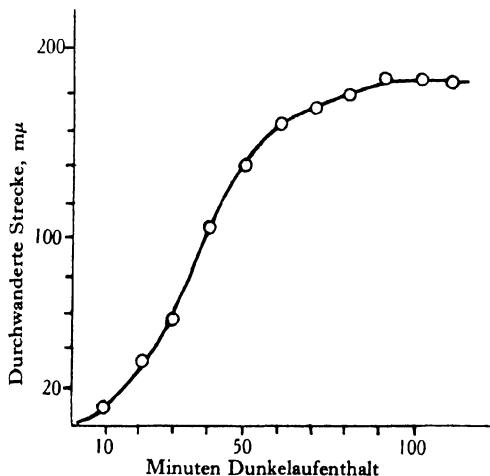
Fig. 3. Dunkeladaptation von *Palaemonetes vulgaris*
gemessen an der Pigmentwanderung. (Nach J. H.
Welsh, 1930a.)
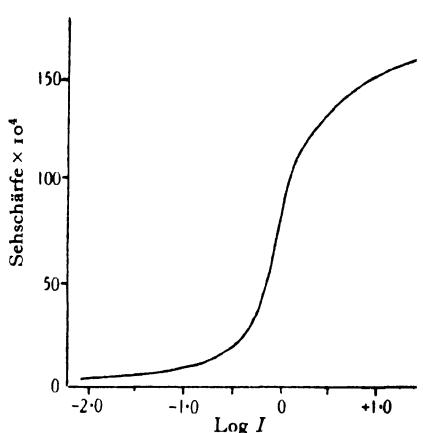
Fig. 4. Abhängigkeit der Sehschärfe der Biene
von der Lichtintensität. (Nach Hecht.)

Sie reagierten erst, wenn sich bei der Lichtkompassorientierung der Lichtstrahl um
den dreifachen Betrag des Ommatidienwinkels verschob, während sie im hellen
Licht schon auf die Veränderung der Richtung um einen Ommatidienwinkel
scharf ansprachen (vgl. auch S. 303). Die Autoren haben hieraus gefolgert, dass bei
schwacher Beleuchtung eine centrale Koppelung von je 7 Ommatidien zu einer
neuen Einheit eintritt.

Einem ähnlichen Phänomen waren schon früher Hecht und Wolf (1929) bei
ihren vielbeachteten und oben besprochenen Sehschärfeprüfungen der Honig-
biene begegnet, aber ihre Deutung ist eine vollständig andere. Hecht und Wolf
(1929) fanden mit Hilfe einer sehr raffinierten Technik, dass die Sehschärfe der
Biene ganz ähnlich wie die des Menschen mit der Beleuchtung abnimmt (Fig. 4).
In Anlehnung an gewisse Hypothesen, die früher für das menschliche Auge

entwickelt wurden, schliessen sie hieraus, dass auch beim Bienenauge die einzelnen Ommatidien ganz verschiedene Reizschwellen besitzen sollen. Die Zahl der funktionstüchtigen Ommatidien wächst also mit der Belichtungsstärke, dementsprechend nimmt ihre gegenseitige Entfernung ab und die hiervon abhängige Sehschärfe zu. Die Hechtsche Hypothese ist zur Zeit nicht widerlegt, obgleich eine experimentelle Prüfung mit anderen Mitteln wohl möglich wäre. Sicher ist aber, dass das beobachtete Phänomen sich bei Mensch und Biene auch ganz anders erklären liesse. Man braucht nämlich auch hier nur anzunehmen, dass im Masse, wie die Belichtung schwächer wird, immer mehr benachbarte Ommatidien zu einer neuen funktionellen Einheit durch Ableitung zu einer einzigen Nervenzelle zusammengeschlossen werden. Diese Hypothese besitzt ohne Zweifel den Vorzug, dass im Auge keine "toten Räume" entstehen, die überhaupt keinen Lichtreiz bei zu schwacher Belichtung percipieren.

Eine andere Erscheinung, die mit der Frage der centralen Adaptation wahrscheinlich zusammenhängt, fand Dolley (1929). Nach seinen Untersuchungen bedingt bei *Eristalis tenax* Aufenthalt im Dunkeln, dass die Lichtempfindlichkeit binnen einer Stunde auf etwa das 20-fache ansteigt. Lässt man das Tier noch länger im Dunkeln, so verändert sich die Empfindlichkeit in den nächsten zwei Stunden nicht, nimmt aber dann rapide ab, und sinkt unter die Werte, die für das helladaptierte Auge gelten.

Es ist selbstverständlich, dass diese Erscheinung nicht mit den physikochemischen Processen in der Sinneszelle erklärt werden kann. Dagegen ergeben sich hier offenbar Beziehungen zu der bei manchen anderen Tieren festgestellten Unerregbarkeit nach längerem Dunkelaufenthalt. Der Schreiber dieser Zeilen hat bei dem cirripeden Krebs *Balanus* sowie bei den Borstenwürmern *Hydroides* und *Branchiomma* schon vor einigen Jahren gezeigt, dass nach längerer Verdunkelung die sonst sehr empfindlichen Tiere eine Zeitlang auf gar keinen Lichtreiz mehr reagieren. Die Erregbarkeit der Centren ist also durch den Wegfall des Lichtreizes bis auf Null gesunken. Vielleicht liegen bei *Eristalis* ähnliche Verhältnisse vor.

Die hier geschilderte centrale Adaptation des Facettenauges, die sich in einer Änderung der Sehschärfe bei wechselnder Belichtung äussert, gilt aber keineswegs allgemein. Beim Ohrwurm (*Forficula*) fanden Buddenbrock und Schulz (1933) gar keine solche Abhängigkeit, und das Gleiche hat ganz neuerdings Bröker (1934) bei *Eupagurus* gefunden. Natürlich können aber auch diese Tiere sehr wohl helleres und dunkleres Licht unterscheiden. So antwortet *Eupagurus* bei geringer Lichtintensität mit schwächeren Antennenbewegungen als bei starkem Licht.

Neben der physiologischen Adaptation kann man auch beim Arthropodenauge eine physikalische unterscheiden, die sich in einer Wanderung der umhüllenden Pigmente äussert. Die ausgiebigsten derartigen Wanderungen sind bei den sogenannten Superpositionsaugen anzutreffen, die sich hierdurch im hellen Licht in Appositionsaugen verwandeln können. Im typischen Falle, wie er sich etwa beim Flusskrebs findet, sind zwei gänzlich gesonderte Pigmente zu unterscheiden, ein proximal gelegenes Retinapigment, das sich in den Retinazellen selbst findet, und ein distales Irispigment in besonderen Pigmentzellen. Im Dunkeln weichen beide

Pigmente auseinander, sodass der gesamte Mittelbezirk des Ommatdiums von Pigment völlig frei wird. Im Hellen tritt die entgegengesetzte Bewegung ein. Die Mechanik der Pigmentwanderung ist in den meisten Fällen eine Körnchenströmung im Innern der unbeweglichen Zelle. Jedoch ist für gewisse Krebse (*Palaemonetes*, Welsh, 1930 a) auch das Vorhandensein muskulärer Fibrillen beschrieben worden, deren Kontraktion ein sich Zurückziehen des ganzen Zelleibes bedingt (Fig. 5).

Im Vergleich zu diesem Beispiele erleidet die Pigmentwanderung in vielen anderen Fällen eine mehr oder weniger grosse Reduktion. So ist bei dem Nachtfalter *Porthesia* zwar das distale Pigment beweglich, das Retinapigment hat aber seine Beweglichkeit eingebüsst. Bei manchen Tagfaltern, die in teilweise schattigem Gelände fliegen, ist nach Demoll (1917) noch ein Rest von Pigmentverschiebung in den Retinazellen wahrzunehmen, während die distalen starr geworden sind. Endlich ist bei den typischen Appositionsaugen (die meisten Taginsekten, aber auch gewisse Krebse wie *Eupagurus*) jede Pigmentwanderung verschwunden.

Fig. 5. Ommatidien von *Palaemonetes vulgaris*. D, Dunkelstellung; H, Hellstellung, links mit Pigment, rechts entpigmentiert; F, contraktile Fibrillen. (Nach Welsh, 1930 a.)

Die Pigmentwanderung des Arthropodenauges zeigt einen ausgesprochenen Tag- und Nachtwechsel, der auch bei konstanten Beleuchtungsbedingungen zäh festgehalten wird. Dies ist für die Krebse *Cambarus* und *Macrobrachium* festgestellt worden (Welsh, 1930 b; Bennitt, 1932 a) sowie für gewisse Nachtfalter. Von Interesse ist ferner, dass nach neueren Untersuchungen von Bennitt (1932 b) bei *Palaemonetes* eine gegenseitige Beeinflussung beider Augen nachweisbar ist (s. Tabelle II).

Tabelle II.

	Dunkelstellung	Intermediär	Lichtstellung
Beide Augen verklebt	1 Auge	28 Augen	13 Augen
Ein Auge verklebt	0 Auge	9 Augen	19 Augen

Im zweiten Falle wirkt sich die Belichtung des freigebliebenen Auges dahin aus, dass ein grösserer Procentsatz der verklebten in Lichtstellung übergeht.

Neben den verhüllenden Pigmentzellen ist in den Augen vieler Arthropoden auch ein Tapetum vorhanden, das aus lichtreflektierenden Zellen besteht. Auch diese Zellen können Wanderungen ausführen. Bei *Macrobrachium* besteht das reflektierende Pigment aus amorphem Guanin und liegt in einem Netzwerk von Zellen, welche die Basis der Retinazellen umgeben. Im Dunkeln liegt dieses Tapetum distal der Basalmembran, das Retinapigment dagegen proximal. Im Hellen vertauschen beide ihre Plätze. Das reflektierende verschwindet hinter der Basalmembran, während das Retinapigment nach vorn wandert (Welsh, 1932).

II. DIE LEISTUNGEN DES GESAMTEN FACETTENAUGES.

(1) DAS BEWEGUNGSSEHEN.

Schon Exner (1891) hat betont, dass das Facettenauge in besonderem Masse geeignet wäre, Bewegungen wahrzunehmen. Die Richtigkeit dieser Anschauung ist seitdem vielfach bestätigt worden. Für viele Insekten und Krebse darf man wohl den Satz aussprechen, dass sie überhaupt nur auf sich bewegende Dinge ansprechen.

Die biologische Bedeutung des Bewegungssehens kann sehr verschieden sein. Die optisch geleitete Fanghandlung vieler Raubinsekten wird vom Bewegungssehen ausgelöst. Bei diesen Tieren bewirkt die Reizung seitlicher Augenteile eine Kopfdrehung solange, bis der gesichtete Gegenstand in den Sehraum der vorderen Ommatidien kommt und binokular betrachtet werden kann (S. 302). Der Räuber kann dann geradlinig auf die Beute sich zubewegen und in der angemessenen Entfernung zuschlagen. Sich nicht bewegende Beutetiere machen auf solche Raubinsekten gar keinen Eindruck.

Weiter verbreitet ist die Rolle, die das Bewegungssehen beim Erkennen von Feinden spielt. Alle scheuen Insekten, die sich schwer mit der Hand greifen lassen und davonfliegen, wenn man sich ihnen nähert, z. B. Fliegen, Tagfalter und viele andere, reagieren ebenfalls auf die Bewegung. Der stillsitzende Mensch stört sie nicht. Ganz allgemein gilt hierbei, dass der sich nahende Gegenstand von einer gewissen Grösse sein muss, soll er die Fluchtreaktion auslösen. Wahrscheinlich gehört in diese Rubrik auch die von Doflein (1910) entdeckte sogenannte Signalreaktion von *Eupagurus* und manchen anderen Krebsen. Sie folgen einem vor dem Aquarium vorbeigeführten Gegenstande mit den langen zweiten Antennen, mit denen das sich nahende Objekt geprüft wird.

Endlich sei erwähnt, dass bei manchen Fliegen, z. B. der Stubenfliege, das Bewegungssehen im Dienste des Sexuallebens steht. Die Männchen stürzen sich auf jeden Gegenstand von Fliegengrösse, der mit entsprechender Geschwindigkeit durch den Raum bewegt wird. Man kann daher nach v. Üxküll eine Falle für männliche Fliegen konstruieren, indem man eine kleine schwarze Kugel mit Leim bestreicht und an einem Faden durch die Luft zieht. Auch bei den Tagfaltern scheint (nach Ilse, 1932) Bewegung des weiblichen Tieres Vorbedingung zu sein für die Einleitung der Liebesspiele durch das Männchen. Sitzende Weibchen werden nicht beachtet.

Ein besonders eigentümliches Bewegungssehen liegt bei etlichen niederen Tieren vor, die Facettenaugen besitzen. Es finden sich solche bei gewissen Muscheln (*Arca, Pectunculus*) am Mantelrande verteilt sowie bei den Vertretern der Polychätengattung *Branchiomma* an der Spitze der zahlreichen Tentakeln. Geht man am Aquarium vorbei, in dem solche Tiere leben, so ziehen sich die Würmer sofort in ihre Röhren zurück, und die Muscheln schliessen ihre Schalen. Dass es sich hierbei nicht um eine Reaktion auf Beschattung handelt, ist leicht in der Dunkelkammer zu beweisen, wo die gleichen Tiere auf Auslöschen einer Glühbirne nicht ansprechen. Es ist hier aber auch kein reines Bewegungssehen vorhanden wie

bei den Arthropoden. Nimmt man eine schwarze Scheibe auf weissem Hintergrunde oder umgekehrt eine weisse auf schwarzem Grunde, so ist nämlich das folgende zu beobachten: Die Tiere (*Branchiomma*) reagieren auf Annäherung der schwarzen und auf Entfernung der weissen Scheibe. Es kann also behauptet werden, dass zur Auslösung der Fluchtbewegung nötig ist, dass eine Bewegung gesehen wird, die mit einer Verdunkelung einhergeht (Buddenbrock, 1934).

Eine interessante Variante des Bewegungssehens bilden die optomotorischen Reaktionen. In der physiologischen Literatur besonders der letzten Jahre spielen diese Reaktionen eine bedeutende Rolle. Sie beschränken sich keineswegs auf die Arthropoden, sondern finden sich auch bei den Wirbeltieren und äussern sich darin, dass das Tier charakteristische Bewegungen mit den Augen, dem Kopf oder dem ganzen Körper ausführt, wenn seine Umwelt in rotierende Bewegung versetzt wird. Die gewöhnliche zuerst von Radl (1903) angegebene Versuchsanordnung ist die folgende: Das Tier sitzt in einem Glasgefäss im Centrum eines Kreises, an dessen Peripherie verschiedene optische Marken (senkrecht stehende Pappstreifen etc.) angebracht sind. Das Tier reagiert, sobald der Kreis und seine Marken in Bewegung versetzt werden.

Die optomotorische Reaktion kann benutzt werden, um alle möglichen anderen Fragen zu lösen und ist uns schon verschiedentlich begegnet (Sehschärfenprüfung, Unterschiedsempfindlichkeit, u.s.w.). Aber auch das Studium ihrer selbst ist der Mühe wert. Ihre biologische Bedeutung ist noch sehr umstritten. Nach der Meinung des Verfassers, die aber noch keineswegs bewiesen ist, handelt es sich hierbei nicht um eine Reaktion auf reale Bewegungen der Umweltsdinge. Der Unterschied scheint mir darin zu liegen, dass in der normalen Umwelt des Tieres stets nur einzelne Dinge vor dem ruhenden Hintergrunde sich vorbeibewegen, während beim optomotorishen Versuch die gesamte Umwelt in Rotation gerät. In eine ähnliche Situation gerät das freilebende Tier nur dann, wenn es sich um seine eigene Vertikalachse dreht. Es wäre also vielleicht möglich, die optomotorischen Reaktionen mit etwaigen Reaktionen zu vergleichen, mit denen das Tier die retinalen Bewegungen beantwortet, die bei seiner Eigenbewegung auftreten.

Diesem Unterschiede entsprechend reagiert z. B. der Einsiedlerkrebs *Eupagurus* auf das Vorbeibewegen einzelner grösserer Objekte mit gleichsinnigem Mitbewegen seiner zweiten Antennen, auf Drehung der Trommel im optomotorischen Versuch dagegen durch Mitbewegen der Augenstiele. Beide Reaktionen sind also *grundsätzlich* verschieden. Dies würde kaum der Fall sein, wenn der Situationsunterschied für das Tier nur darin gelegen wäre, dass das eine Mal *ein* Gegenstand, das andere Mal *mehrere* bis *viele* an seinem Auge vorbeiziehen.

Als Argument dafür, dass die optomotorischen Reaktionen den retinalen Verschiebungen gelten, die durch die Eigenbewegungen des Tieres herbeigeführt werden, kann auch das folgende gelten: *Carcinus maenas*, die gemeine Taschenkrabbe, reagiert nicht nur auf passive, sondern auch auf aktive Drehung um die Vertikalachse deutlich mit kompensatorischen Augenstielbewegungen im Sinne der optomotorischen Reaktionen. Bei der aktiven Bewegung treten aber reale Bewegungen in der Umwelt des Tieres garnicht auf.

Der Einwand, dass die optomotorischen Reaktionen möglicherweise identisch sind mit den Lichtkompassreaktionen (vgl. S. 303), kann durch den Hinweis widerlegt werden, dass diese letzten normalerweise gerade dann auftreten, wenn *ein* sichtbares Objekt das Tier leitet, während die optomotorischen Reaktionen, wie schon mehrfach erwähnt worden, durch *einen* rotierenden Gegenstand niemals auszulösen sind.

Dass man die Dinge aber auch ganz anders anschauen kann, bezeugen die Ausführungen von Hertz (1934), die geradezu den Beweis erbringt, dass ihre Versuchstiere (Stubenfliegen) reale Umweltsbewegungen von den retinalen Bewegungen der oben geschilderten Art unterscheiden. Sie benutzt hierzu zwei übereinander angeordnete Streifencylinder, von denen der untere feststeht. Das Tier reagiert sehr prompt auf Drehung des oberen, läuft also mit, ungeachtet des Umstandes, dass die Streifen des unteren Cylinders dabei retinale Bewegungen hervorrufen. Weitere Untersuchungen auf diesem schwierigen Gebiet sind jedenfalls abzuwarten.

Systematische Untersuchungen über die optomotorischen Reaktionen sind in letzter Zeit unabhängig von einander von verschiedenen Autoren durchgeführt worden (Buddenbrock u. Friedrich, 1933; Gaffron, 1933; Hertz, 1934). Trotz der sehr grossen Verschiedenheit der Versuchstiere (*Carcinus* u. *Pollenia* (Stubenfliege)) zeigte sich übereinstimmend, dass der optimale Effekt dann eintritt, wenn die sich bewegenden Objekte (senkrechte, schwarze Pappstreifen) gleichmässig über den ganzen Gesichtskreis verteilt sind, wobei zu berücksichtigen ist, dass der Gesichtskreis der untersuchten Tiere häufig 360 Grad beträgt. Bewegungen eines einzigen noch so breiten Streifens bleiben dagegen wirkungslos. Hiermit hängt es zusammen, dass es hemmend wirkt, wenn die rotierenden Streifen nur in einem Teile des Gesichtsfeldes sichtbar sind. Nimmt man zur Abblendung der Streifen nur einen unbeweglichen Sektor, so verschwinden die Reaktionen bei der Stubenfliege, wenn dieser Sektor 180 Grad übersteigt, bei *Carcinus*, ist erst bei 300 Grad die Grenze erreicht. Mehrere bis viele kleinere Sektoren, die im Umkreise verteilt stehen, können bei der Fliege zusammen bis 270 Grad umfassen, ohne dass die Reaktion erlöscht. Zur Beurteilung des ganzen Verhaltens ist es auch wichtig, dass ruhende Konturen, die dem Auge des Tieres gleichzeitig dargeboten werden, den Effekt mehr oder weniger hemmen. So heben vier in Oppositionsstellung befindliche unbewegt schwarze Streifen bei der Fliege die Wirkung des rotierenden Cylinders völlig auf.

Von Wichtigkeit für die Theorie des Bewegungssehens ist die von Gaffron (1933) an der Stubenfliege *Pollenia* gemachte Beobachtung, dass es hier keine stroboskopischen Scheinbewegungen gibt. Sprunghaft sich ändernde optische Reize, die also nicht nach einander die benachbarten Ommatidien 1, 2, 3, 4, 5·· ergreifen, sondern etwa nur 1, 4, 7 während 2, 3 und 5, 6 ungereizt bleiben, haben keine Reaktion der Fliege zur Folge. Es muss hieraus auf eine principiell andere Verknüpfung der subretinalen Ganglienzellen als bei den Wirbeltieren geschlossen werden; bestimmte Vorstellungen hierüber zu entwickeln, scheint mir indessen noch verfrüht zu sein.

Durch diese Beobachtung Gaffrons ist auch sichergestellt, dass es sich bei der Fliege um ein echtes Bewegungssehen handelt und nicht um eine modificierte Lichtkompassbewegung, denn bei dieser (vgl. S. 303) sind gerade sprunghafte Veränderungen der gereizten Retinastelle bewegungsauslösend.

Von grossem Interesse ist die Tatsache, dass neuerdings auch der Nachweis inducierter Objektbewegung bei Insekten gelungen ist. Man versteht hierunter die scheinbaren Bewegungen ruhender Gegenstände, die man wahrzunehmen glaubt, wenn andere Teile des Gesichtsfeldes sich in tatsächlicher Bewegung befinden. Gaffron (1933) konnte zeigen, dass Aeschnalarven nach unbewegten Objekten (Holzkugeln von 0·4 cm. Durchmesser) schnappen, wenn hinter ihnen der häufig erwähnte Streifencylinder vorbeibewegt wird. Mit derselben Methode gelang der gleichen Autorin auch der Nachweis der Nachwirkung von Bewegungen. Wird der Streifencylinder längere Zeit, etwa 10 Minuten hindurch, gedreht und dann plötzlich abgedeckt, so schnappen die Larven auch jetzt nach ruhenden Objekten von geeigneter Grösse, wenn sie zufällig in die Fixierrichtung des Tieres kommen.

Das Sehen von bewegten Objekten ist bei den Facettenaugen genau so wie beim Wirbeltierauge abhängig von der Bewegungsgeschwindigkeit (Frequenz) und der Beleuchtungsintensität. Die maximalen Frequenzen, bei denen das Tier bei optimalem Licht die Bewegung noch erkennt, sind fast die gleichen wie beim Menschen: *ca.* 56 p. Sek. bei der Libellenlarve (Sälzle, 1932) und dem Einsiedlerkrebs (Bröker, 1934 unveröff.) gegen 70 beim Menschen. Trägt man im Coordinatensystem die Frequenz in Bezug auf den Logarithmus der Belichtungsintensität ein, so erhält man merkwürdigerweise bei beiden Tieren sehr Verschiedenes, bei *Eupagurus* eine gerade Linie, bei *Aeschna* eine S-förmige Kurve. Worauf diese auffälligen Unterschiede beruhen, ist noch nicht bekannt.

(2) DIE REAKTIONEN AUF BELICHTUNG BESTIMMTER AUGENTEILE.

Das Facettenauge der Arthropoden ist keine Summe nebeneinander geschalteter gleichwertiger Ommatidien. Schon die Stellung der verschiedenen Ommatidien zur Hauptachse des Körpers und damit zum Lebensraume des Tieres bedingt physiologishe Verschiedenheiten. Dementsprechend kann man bei vielen Formen (untersucht sind hauptsächlich gewisse Insekten) feststellen, dass der Reizung bestimmter Ommatidien ganz bestimmte Reflexe zugeordnet sind. Hierbei zeigt es sich, dass selbst bei einem und demselben Tier die Reflexe, die von einer bestimmten Augenstelle aus auslösbar sind, je nach dem physiologischen Gesamtzustande sich ändern, in dem sich das Insekt befindet. So lassen sich bei den Fliegen mindestens drei derartige Situationen unterscheiden: Ruhe, phototaktische Stimmung, Lichtkompasstimmung. Das Tier ist also in der Lage, in seinem Gehirn gewisse Umschaltungen vorzunehmen, durch welche die einzelnen Ommatidien bald in diesen, bald in jenen Reflexbogen eingeschaltet werden.

(a) Die tonischen Reflexe.

In der Ruhelage treten die tonischen Reflexe hervor. Sie äussern sich darin, dass das Tier unter der Einwirkung des Lichtes eine ganz bestimmte Haltung einnimmt. Deutlich wird dies allerdings erst, wenn man Teile eines oder beider Augen durch Überlackieren ausschaltet. Am meisten sind Fliegen untersucht. Garrey (1918) wies nach, dass *Proctacanthus* nach Zukleben eines Auges eine charakteristisch schiefe Stellung einnimmt, bei der das sehende Auge dem Boden zugekehrt ist, und auch der Körper eine entsprechende Neigung aufweist. Verkleben der unteren Augenhälften bewirkt, dass sich das Tier vorn steil aufrichtet. Verkleben der oberen Partieen, dass es den Kopf tief nach unten beugt. Etwas weiter haben dann die Untersuchungen von Mast (1923) geführt, der am gleichen Tier die folgenden

B   A
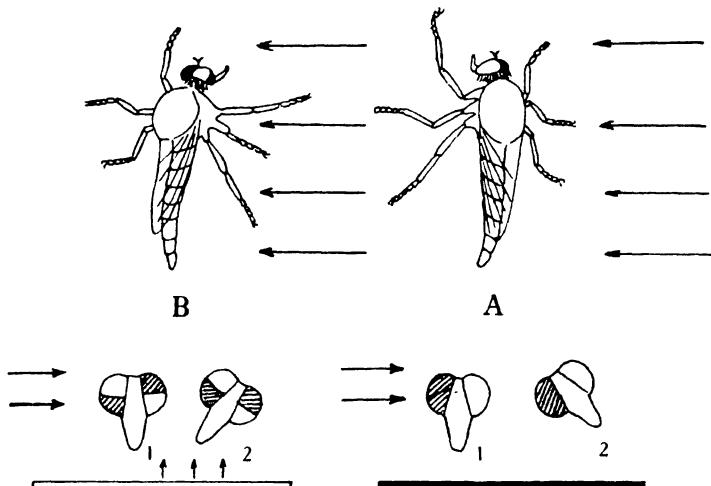
Fig. 6. *Proctacanthus*. Haltung im horizontalen Licht bei teilweiser Verklebung der Augen auf weissem und schwarzem Grunde. (Nach Mast, etwas verändert, 1923.)

Merkwürdigkeiten aufdeckte. Unter dem Einfluss eines horizontalen Lichtbündels dreht das rechts geblendete Tier auf schwarzem Untergrunde dem Licht den Rücken zu. Verklebt man aber die untere Hälfte des rechten und die obere des linken Auges, so dreht das Tier auf weissem Untergrunde die ventrale Kopfhälfte dem Lichte zu (Fig. 6). Diese und ähnliche Beobachtungen führen zu dem Schluss, dass die normale Haltung des Tieres die Resultante zahlreicher Reflexe ist, die von den verschiedenen Augenteilen ausgehen. Die Reizung gewisser peripherer Ommatidien bewirkt offenbar eine Reflexbewegung, die das Licht anderen mehr central gelegenen zuführt. Die Haltungen in Fig. 6 erklären sich demnach wie folgt. In der Situation A_1 ist die einzige stärker belichtete Partie die dorsale Innenfläche des linken Auges. Die Reizung der dort befindlichen Ommatidien führt zu einer Drehung bis zur Stellung A_2, in der die direkt dorsal gerichteten Ommen vom Lichte getroffen werden. In Situation B_1 überwiegt die Wirkung des linken Auges

wegen der viel grösseren Empfindlichkeit der ventralen Ommen. Der Reflex
führt auch hier dazu, dass die mehr centralen, unmittelbar ventral schauenden
Einzelaugen den Lichtreiz empfangen. Die Schiefhaltung des Körpers und der
Beine ist in allen solchen Fällen sicherlich von Reflexen abzuleiten, deren Aufgabe
es ist, die normale Lagebeziehung zwischen Kopf und Körper möglichst wiederher-
zustellen. Zwischen den eigentlichen Augenreflexen und diesen Kopf-Körper-
reflexen wird dann gewissermassen ein Kompromiss geschlossen.

(b) Der Einfluss des Lichts auf die Aktivität der Tiere.

Ebenso wie die Haltung des Insekts durch das Licht mitbedingt wird, ist das
Licht von Einfluss auf die allgemeine Aktivität der Arthropoden. Es ist wahr-
scheinlich, dass auch hierbei die verschiedenen Augenteile eine unterschiedliche
Rolle spielen. Näheres hierüber ist allerdings noch nicht bekannt. Misst man die
Geschwindigkeit, mit der ein solches Tier eine bestimmte Strecke unter sonst glei-
chen Umständen zurücklegt, so ergibt sich eine deutliche Abhängigkeit von der
Lichtintensität. Als Beispiel sei das Verhalten des Käfers *Popillia japonica* (nach
Moore u. Cole, 1921) angeführt.

Tabelle III.

Lichtintensität in Meterkerzen	Zeit für Durch-Zurücklegung einer Strecke von 12 5 cm in
85	15 50
234	13 14
608	11 60
1600	10 86
3276	10 22

Eine derartige photokinetische Wirkung ist auch für Cladoceren (*Leptodora hyalina*)
beschrieben worden, deren rhythmische Vertikalbewegungen höchstwahrscheinlich
von der Lichtintensität abhängen. Quantitative Untersuchungen scheinen aber
hierüber noch nicht vorzuliegen.

Kropp und Enzmann (1933) stellten neuerdings fest, dass auch die Zahl und die
Amplitude der Beinbewegungen des frei aufgehängten Flusskrebses vom Lichtreiz
abhängen. Bei einseitig geblendeten Tieren ist der Unterschied der Bewegungen
auf beiden Seiten beträchtlich.

(c) Der Lichtrückenreflex.

Den geschilderten Reflexen ist endlich auch der sogenannte *Lichtrückenreflex*
anzuschliessen (v. Buddenbrock, 1914). Er ist zwar keine specifische Eigentüm-
lichkeit der mit Facettenaugen versehenen Arthropoden, aber doch in dieser Tier-
gruppe besonders weit verbreitet. Er findet sich hier bei Insekten und Krebsen und
besteht darin, dass diese Tiere besonders während des Schwimmens dem von oben
einfallenden Lichte den Rücken (oder den Bauch) zukehren. Das Licht wird also
mit den dorsalen Ommatidien (seltener mit den ventralen) aufgefangen, bezw.
ihnen zugeleitet.

Der Lichtrückenreflex ist eine wichtige Komponente bei der Gleichgewichtserhaltung schwimmender Tiere. Er kann allein auftreten (Ephemeridenlarven, Dytiscidenlarven, manche Mysideen und Garnelen), er kann synergistisch mit statischen Reflexen gekoppelt sein (die meisten Garnelen u. a. mit Statocysten versehene schwimmende Dekapoden). Er kann endlich auch ganz fehlen. Dies ist z. B. bei den vermöge ihrer Luftbehälter im stabilen Gleichgewichte schwimmenden Corethralarven der Fall oder bei gewissen mit Statocysten versehenen Krebsen wie *Penaeus*. Für eine Reihe von Fällen ist nachgewiesen, dass ein Auge allein genügt, um die richtige Lage zu erzwingen (*Processa caniculata, Palaemon,* u. a.). Alverdes, 1926.

Hieraus ergibt sich, dass der Lichtrückenreflex keineswegs immer auf einer Gleichgewichtsreaktion, einer sogenannten Tropotaxis beruht. Es folgt dies auch daraus, dass kurze, gedrungene Tiere bei plötzlicher Unterbeleuchtung einen Purzelbaum und keine Drehung um die Längsachse ausführen.

Bei den mit Statocysten versehenen Tieren wird der Lichtrückenreflex normalerweise durch die Statoreflexe überdeckt und ist nicht nachweisbar. Belichtet man z. B. eine schwimmende Garnele oder eine *Mysis* von unten, so schwimmt das Tier gleichwohl in der Bauchlage, da die Statocysten diese Lage gegen die Wirkung des Lichtrückenreflexes erzwingen. Erst, wenn beide Statocysten entfernt sind, tritt der Lichtrückenreflex im Experiment klar zu Tage: das von oben belichtete Tier schwimmt in Bauchlage, das von unten belichtete in Rückenlage.

Bei den Garnelen (*Leander*) ist, wie Alverdes (1926) nachgewiesen hat, der Lichtrückenreflex auch während des Sitzens vorhanden, was seine Verwandtschaft mit den tonischen Reflexen erweist, er kann nur nicht zur Geltung kommen, weil er durch die Statocysten gehemmt wird. Sobald aber die Statocysten entfernt sind, treten diese Reflexe in aller Deutlichkeit hervor. Den Beweis hierfür liefert der Vergleich des auf schräger Unterlage sitzenden normalen und entstateten *Leander* (s. Fig. 7 *a*, *b*). Das normale Tier sitzt völlig symmetrisch zu seiner Unterlage. Es ist weder Rücksicht genommen auf den Lichteinfall noch auf die Schwerkraft. Das bedeutet, dass Stato- und Lichtreflexe gehemmt sind. Die Statoreflexe erleiden ihre Hemmung, wie wir wissen, durch die von den Füssen ausgehenden Tangoreflexen, und man könnte zunächst annehmen, dass für die Lichtreflexe dasselbe gilt. In diesem Falle dürfte aber in der Sitzweise des normalen und des entstateten Krebses kein Unterschied sein. Dass ein solcher existiert (s. Fig. 7*a*, *b*) beweist, dass die Lichtreflexe normalerweise durch die Statocysten gehemmt werden.

Den Anforderungen des Lichtrückenreflexes wird bei den stieläugigen Dekapoden (Garnelen) zunächst durch die Bewegungen der Stielaugen selbst Genüge getan. Bringt man einen freischwebenden *Leander* in Schieflage (Fig. 7*c*), so vollführt er mit Hilfe bestimmter Beinbewegungen eine Drehung, die ihn in die Normallage zurückführt. Bevor aber dies geschieht, ist als Erstes eine Wendung der Augenstiele nach der Richtung zu beobachten, nach der die Wendung ausgeführt wird. Das heisst, die Augenstiele suchen sich, soweit es geht, so einzustellen, dass sie die normale Lage zum Lichte einnehmen. Es ist dies auch bei entstateten Tieren nachzuweisen (Alverdes, 1926).

Von besonderem Interesse ist, dass die Augenstielreflexe die Ursache sind für

das Eintreten der sogenannten Augenstielkörperreflexe, deren Aufgabe es ist, die normale Stellung der Augenstiele zum Körper wiederherzustellen. In dem soeben geschilderten Falle führt dieser Reflex zur Weiterdrehung des Tieres, bis es die Normallage erreicht hat, bei der der Rücken dem Lichte zugekehrt ist und die Augenstiele ihre gewöhnliche Stellung zum Körper haben. Besonders lehrreich für die Erkenntnis dieser wichtigen Reflexe ist auch das Verhalten des einäugigen
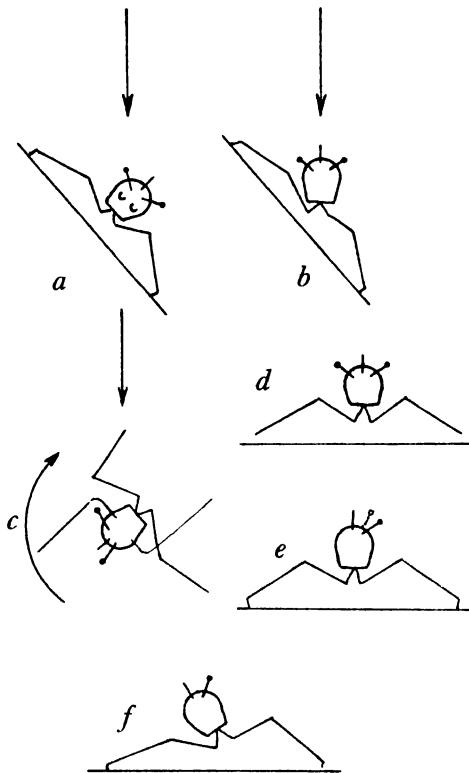
Fig. 7. Haltung und Augenstellung einer Garnele unter verschiedenen Bedingungen. Näheres im Text. (Nach Alverdes, 1926.)

Krebses. Setzt man einen einäugigen Krebs (*Leander*) auf eine horizontale Unterlage und beleuchtet in diffus von oben, so neigt er zunächst den noch vorhandenen Augenstiel dem Lichte zu (Fig. 7 e), während die Stellung des Auges zum Körper eine falsche wird. Der jetzt einsetzende Augenstielkörperreflex verursacht dann, dass sich der Körper schief neigt, bis zu einer Stellung, wie sie Fig. 7 f angibt.

Dieser Versuch zeigt gleichzeitig, dass sich der zweiäugige Krebs in entsprechender Lage (Fig. 7 g) keineswegs in einem optisch reizlosen Zustande befindet. Zwischen beiden Augenstielen besteht eine latente Spannung. Jeder Augenstiel sucht sich nach Möglichkeit dem Lichte zuzudrehen und wird nur durch eine Gegenwirkung des anderen Auges daran gehindert.

(d) Die Phototaxis.

Die Phototaxis ist eine Erscheinung, die bei den mit Facettenaugen versehenen Arthropoden ausserordentlich verbreitet ist. Man kann drei Arten unterscheiden, die positive Ph., die negative Ph. und die Skototaxis. Bei der positiven Ph. wird der leitende Lichtstrahl den nach vorn gerichteten Ommatidien zugeleitet und darin festgehalten, sodass das Tier geradlinig auf das Licht zuläuft. Bei der negativen Ph. wird der Lichtstrahl umgekehrt den hintersten Ommatidien zugeführt, bei der Skototaxis, die das Tier ins Dunkle führt, werden dunkle Flächen oder Gegenstände mit den vorderen Ommatidien visiert.

Über das Zustandekommen dieser verschiedenen Einstellungsarten sind die Meinungen der Forscher noch geteilt. Die älteste Hypothese ist die Tropismenhypothese von Loeb (1913), die das Phänomen der gerichteten Einstellung zum Licht hauptsächlich auf den bilateralen Körperbau und die durch ihn bedingte Erregungssymmetrie zurückführt, ohne die Annahme besonderer Reflexe. Es wird angenommen, dass die verschiedene Belichtung der beiden Augen bei Lichteinfall von der Seite die Ursache dafür ist, dass das Tier rechts und links verschieden starke Bewegungen ausführt, bis es zu einer symmetrischen Einstellung zum Lichte gelangt. Sobald dies geschehen ist, liegt kein Grund vor zu einer weiteren Drehung, das Tier bewegt sich daher geradlinig auf das Licht zu. Für und gegen die Tropismenlehre ist bis in die neueste Zeit sehr viel geschrieben worden, und es würde zuweit führen, die einzelnen Argumente hier zu beleuchten. Es genüge die Bemerkung, dass jedenfalls nur ein Teil der bei der Phototaxis zu beobachtenden Erscheinungen durch die Tropismenlehre erklärbar ist.

Eine von der Tropismenlehre leicht abzuleitende Folgerung ist, dass einäugige Tiere sich ständig im Kreise bewegen, also sogenannte Circusbewegungen ausführen müssen, wobei je nach der Art der Phototaxis das Tier entweder dauernd nach der sehenden oder nach der geblendeten Seite abweicht. Dies ist auch, wenngleich es keine allgemeingültige Erscheinung ist, besonders bei diffusem Licht, häufig zu sehen (vgl. Fig. 9); jedoch kann hierfür, wie Mast (1923) überzeugend nachwies, auch eine ganz andere Erklärung angenommen werden. Dieser Forscher betont, dass von jedem Augenteil besondere Reflexe ausgehen, und führt die gesamte Phototaxis auf ein System solcher Reflexe zurück. Sie sind hauptsächlich an Fliegen studiert worden. Die Effektoren sind die Beine. Ihre Stellung und Bewegung ist principiell verschieden, je nachdem, welche Retinastelle eine Reizung erfährt. Wenn bei *Eristalis tenax* oder bei *Erax* der Lichtstrahl die hinterste Partie des Auges trifft, so bewegen sich die Füsse der einen Seite vorwärts, die der anderen rückwärts (Fig. 8). Bei seitlicher Reizung bewegen sich beide Vorderbeine auf das Licht zu. Werden die nach vorn gerichteten Ommatidien vom Lichte getroffen, so machen beide Vorderbeine eine Vorbewegung, die das geradlinige Hineilen des Tieres zum Licht einleitet. Endlich kann man den Lichtstrahl in den vorderen Innenwinkel des Auges schicken. Jetzt erfolgt durch geeignete Beinbewegungen eine entgegengesetzte Drehung des Tieres.

Die Manegebewegungen des einseitig geblendeten Tieres lassen sich nun nach

Mast leicht verstehen, wenn man annimt, dass bei diffusem Licht alle diese Reflexe am sehenden Auge dauernd sich abspielen. Da die Wirkung der seitlichen und hinteren Augenteile, die bestrebt sind, den Lichtreiz den vorderen Ommatidien zuzuführen, überwiegt, ist eine andauernde Drehung des Tieres die notwendige Folge.
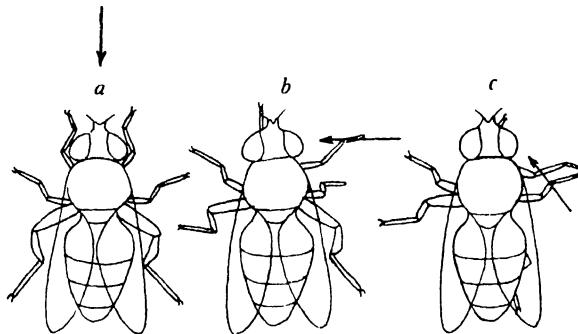
Fig. 8. *Eristalis tenax.* Beinbewegungen nach Reizung verschiedener Augenteile. Pfeil = Lichteinfall. (Nach Mast, 1923.)
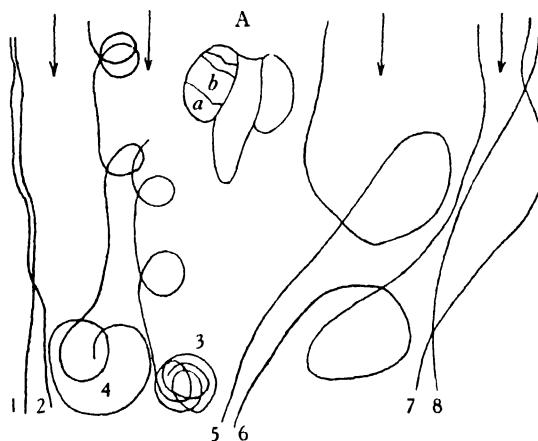
Fig. 9. *Proctacanthus.* A, Kopf von vorn. Die übrigen Figuren Laufspuren bei horizontalem Lichteinfall: 1, 2, normales Tier; 3, 4, links geblendet; 5, 6, ebenso, dazu Abdeckung des ventralen Teiles *a* des rechten Auges; 7, 8, ebenso, dazu Abdeckung der Partieen *a* und *b*. (Nach Mast, 1923.)

Aus dem Vorhandensein der von Mast beschriebenen Reflexe ist ferner ohne weiteres abzuleiten, dass unter der Wirkung einer eng begrenzten Lichtquelle (Sonne, Glühbirne, etc.) häufig keine Manegebewegungen gemacht werden, sondern das Tier den Lichtstrahl dauernd in den vorderen Ommatidien festhält und so auch einäugig zum Lichte gelangt. Man hat diese Erscheinung, bei der das Tier gewissermassen auf ein Ziel zuläuft, auch Telotaxis genannt (Kühn, 1919).

Dass die einzelnen Partieen des Facettenauges in ungleichem Masse an der Phototaxis beteiligt sind, geht auch aus Beobachtungen hervor, die Mast (1924) und

seine Schüler an verschiedenen Insekten gemacht haben. Aus ihnen ergibt sich, dass die nach unten gerichteten Ommatidien, die das vom Boden reflektierte Licht aufnehmen, an der Erzeugung der Circusbewegung einäugiger Tiere besonders beteiligt sind, wie wir dies auch bei den tonischen Reflexen sahen. So verschwinden bei der Fliege *Proctacanthus* die Circusbewegungen, wenn ausser dem linken die unteren Partieen des rechten Auges verklebt werden (Fig. 9).

Ähnlich liegen die Dinge bei dem von Clark (1931) untersuchten Käfer *Dineutes*. Der einseitig geblendete Käfer vollführt bei horizontalem Lichteinfall zunächst starke Circusbewegungen. Nach einiger Zeit verschwinden dieselben und das Tier läuft schräg geradeaus durch das Lichtfeld (Fig. 10). Wird jetzt eine plötzliche Verringerung des von unten einfallenden Lichtes herbeigeführt, so wendet sich das Tier in scharfem Winkel der Lichtquelle zu (Fig. 10*c*); wird das Licht verstärkt, so wendet es sich von der Lichtquelle ab (Fig. 10*d*).
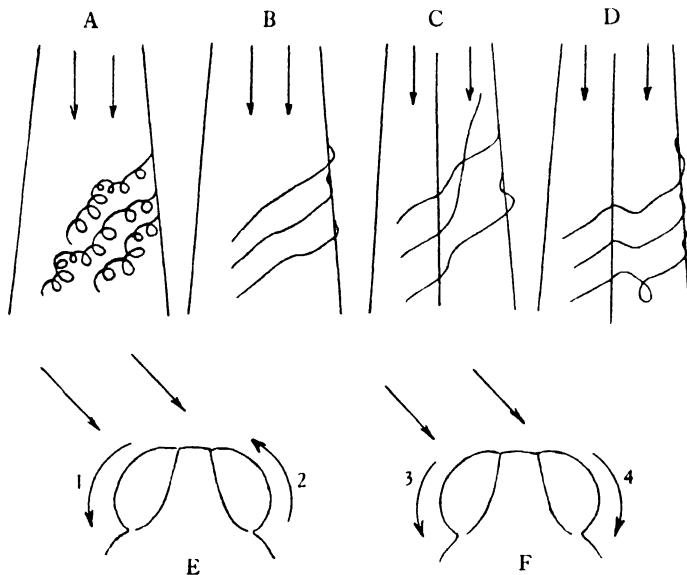
Fig. 10. *Dineutes assimilis*, Laufspuren links geblendeter Tiere. A, sofort nach Einsetzen in das Lichtfeld auf grauem Grund; B, nach mehreren Minuten Aufenthalt im Lichtfeld; C, nach plötzlicher Verstärkung; D, nach plötzlicher Verringerung des vom Boden reflektierten Lichtes im Moment des Überschreitens der Mittellinie; E, schematische Darstellung der reflektorischen Drehungen unter dem Einfluss schräg auffallenden Lichtes, E für die dorsalen, F für die ventralen Facetten. (A–D, nach Clark, 1931.)

Diese auf den ersten Blick hin etwas verwirrenden Beziehungen lassen sich leicht erklären durch die Annahme, dass die von den einzelnen Augenteilen ausgelösten Reflexe sich so verhalten wie Fig. 10*e, f* sie darstellt. Von den seitlichen und dorsalen Augenteilen gehen Reflexe aus, die das Tier dem Licht zuwenden (1 u. 2); von den ventralen, die das vom Boden reflektierte Licht empfangen, beiderseits entgegengesetzte (3 u. 4). Wird jetzt das linke Auge verklebt, so bleibt ein Widerstreit zwischen 2 und 4 bestehen. Im Anfang des Versuches auf weisser

Unterlage oder auf Glasplatte mit durchfallendem Licht überwiegt 4, daher die Circusbewegungen. Sind die ventralen Elemente helladaptiert, so tritt zwischen 2 und 4 eine Art Gleichgewichtszustand ein, das Tier läuft tropotaktisch geführt, geradeaus. Änderung der von unten kommenden Lichtmenge muss dann den beobachteten Erfolg der Abbiegung nach der einen oder nach der anderen Seite haben.

Die Skototaxis, deren Vorkommen bei Gliedertieren lange Zeit bezweifelt worden ist, unterscheidet sich von der positiven Phototaxis wohl nur dadurch, dass der Reiz nicht durch ein Licht, sondern das Fehlen eines solchen, also durch Dunkelheit ersetzt ist. Befindet sich also im Gesichtsfeld des Tieres ein dunkler Gegenstand, so wird sein Bild durch geeignete Bewegungen den vorderen Ommatidien zugeleitet. Der Reflexablauf ist wahrscheinlich der gleiche. Die Skototaxis kann auch mit einem Auge allein ausgeführt werden. Mueller, 1925.

(e) Die Entfernungslokalisation als Reflex, an dem beide Augen beteiligt sind.

Den Arthropoden fehlt das wichtigste Mittel, dessen sich die Wirbeltiere bedienen, um die Entfernung eines gesehenen Gegenstandes festzustellen, die Accommodation. Sie sind daher allein auf den zweiten auch für uns wichtigen Faktor angewiesen, nämlich das binokulare Sehen. Die Ausmessung des Gesichtsfeldes führt zu der Feststellung, dass die gut sehenden Insekten einen binokularen Sehraum besitzen, der stets in Beziehung steht zu der Ausbildung ihrer Fresswerkzeuge. So liegt, wie zuerst Demoll (1917) zeigte, bei den Lepidopteren der ausgestreckte Rüssel zum grössten Teile im binokularen Sehraum, bei den Libellenlarven das vorstreckbare Mentum, bei den Cicindelalarven die Spitze der Mandibeln.

Die genaue Entfernung des Objekts ergibt sich für das Insekt aus der Lage der gleichzeitig gereizten symmetrisch gelegenen Ommatidien des linken und rechten Auges. Erforderlich hierzu ist, dass sich das Objekt nicht seitlich, sondern vor dem Tiere befindet. Aus diesem Zusammenhange versteht es sich, dass manche hierauf untersuchten Insekten wie Cicindela oder Aeschna (Larve), sobald ein Gegenstand ihre Aufmerksamkeit erregt, sich zunächst symmetrisch zu ihm einstellen. Erst jetzt kann das Tier eine Entscheidung treffen. Bei Cicindela erfolgt, wenn der Schnittpunkt der Achsen der gereizten Ommatidien zu entfernt ist, Hinzulaufen auf die Beute, liegt er nahe genug, Zupacken.

Den Beweis für die Richtigkeit der hier entwickelten Auffassung liefert die partielle Blendung (Baldus, 1926; Friedrichs, 1931). Libellenlarven sowie Cicindelen, denen ein Auge total oder in seiner vorderen Hälfte verklebt ist, greifen beim Beutefang meistenteils daneben; oder schnappen, indem sie sich nur nach der scheinbaren Grösse des monokular gesehenen Objekts richten, aus ganz falschen oft viel zu grossen Entfernungen.

(f) Die Lichtkompassbewegung.

Befindet sich das Insekt in einer Stimmung, in der es den Gesetzen der Lichtkompassorientierung gehorcht, so werden andere Reflexe deutlich.

Die Lichtkompassorientierung ist zum ersten Mal von dem Ameisenforscher Santschi (1911) bei Ameisen entdeckt worden, dessen Beobachtungen von Brun (1914) bei den gleichen Tieren bestätigt und erweitert wurden. Ihre weite, vielleicht allgemeine Verbreitung, nicht nur bei den Insekten, sondern auch bei sehr vielen anderen niederen Tieren wurde erst später erkannt. Ihr Name soll besagen, dass sich das Tier zu den Lichtstrahlen in ähnlicher Weise einstellt wie der Mensch bei Gebrauch des Kompasses. Es wird die Bewegungsrichtung, bezogen auf die Richtung des leitenden Strahls bezw. der Nadel, konstant gehalten. Erzwungen wird hiermit in beiden Fällen eine geradlinige Vorbewegung in bestimmter Richtung.

Physiologisch wird dieser Effekt beim Facettenauge sehr einfach dadurch erreicht, dass das Tier während seiner Bewegung den Lichtstrahl im Ommatidium A festhält. Tritt jetzt eine Veränderung ein, durch welche das Licht dem Ommatidium B zugeleitet wird, so vollführt das Tier eine reflektorische Bewegung, durch die das Licht wieder nach A geleitet wird. Bemerkenswert ist hierbei, dass A und B nicht in dem selben Auge zu liegen brauchen. Die Lichtkompassorientierung hat also, wie hier ausdrücklich bemerkt sei, nichts mit Bewegungssehen zu tun. Das Tier reagiert nicht darauf, dass sich der Lichtstrahl von A nach B verschiebt, sondern darauf, dass B statt A belichtet ist. Man kann ein Tier, das sich gewöhnt hat, in einer bestimmten Einstellung zum Lichte zu kriechen, vom Tisch aufheben, für längere Zeit in eine Schachtel sperren und dann in einer beliebigen Stellung zum Licht wieder auf den Tisch setzen. Die kompensatorische Drehung wird sofort eintreten.

Die Lichtkompassreaktion beruht also auf einer gewissen Gedächtnisleistung; sie wird daher von Kühn (1919) als Menotaxis bezeichnet.

Dass es sich bei dieser eigentümlichen Reaktion um den Ablauf eines Reflexes nicht um eine Verstandeshandlung handelt, geht daraus hervor, dass das Tier die alte Reizsituation stets auf dem kürzesten Wege wiederfindet (Buddenbrock, 1931). Nur wenn A und B um 180 Grad von einander abweichen, ist es unbestimmt, ob die Drehung des Insekts links oder rechts herum erfolgen wird. In jedem anderen Falle wird der kleinere Drehwinkel gewählt. Da bei den Insekten ein mathematisches Verständnis nicht angenommen werden kann, bleibt nur die Möglichkeit eines Reflexes übrig (s. Fig. 11).

Die Lichtkompassreaktion ist bis vor kurzem für eine ziemlich grobe Orientierungsart gehalten worden, mit der sich das Tier nur ungefähr zu orientieren vermöchte. Jedoch konnte unlängst nachgewiesen werden, dass es zur Auslösung der kompensatorischen Bewegung vollauf genügt, wenn der Lichtstrahl von einem Ommatidium ins nächste gelangt (Buddenbrock u. Schulz, 1933). Die Methode des Nachweises ist die folgende. Ungefähr 4 Meter von dem Laufbrett entfernt, auf welchem das Insekt kriecht, befinden sich die Glühlampen L_1 und L_2 (Fig. 11), die abwechselnd eingeschaltet werden. Auf eine solche Umschaltung reagiert der Käfer mit einer Änderung seiner Bewegungsrichtung.

Bei diesen Versuchen ist nun festzustellen dass stets eine positive Reaktion eintritt, wenn Winkel α grösser ist als der Ommatidienwinkel β, ist α aber kleiner, so

zeigt sich, dass der Procentsatz der positiven Reaktionen proportional dem Winkel sich verändert. Eine solche Abhängigkeit erhalte ich nun auch bei der Annahme, dass keine Reaktion eintritt, wenn bei der Umschaltung der Lichter der Lichtstrahl im selben Ommatidium verbleibt. Die Wahrscheinlichkeit hierfür ist umso grösser, je kleiner α ist.
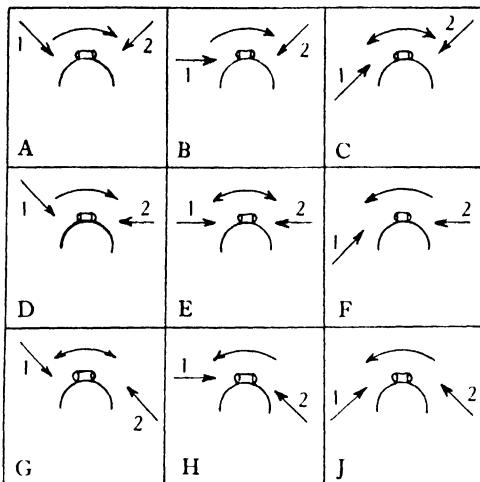
Fig. 11. Lichtkompassreaktion von *Geotrupes*. Umdrehbewegung nach Belichtung des anderen Auges. Pfeil 1, ursprünglicher; Pfeil 2, neuer Lichteinfall. (Nach v. Buddenbrock, 1931.)
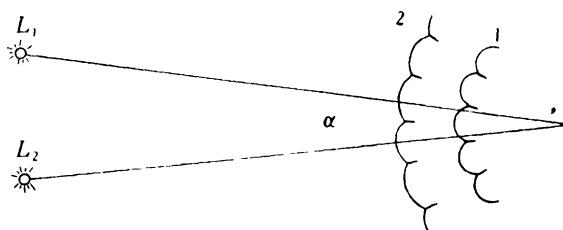
Fig. 12. Schema der Versuchsanordnung zur Prüfung der Lichtkompassbewegung von Insekten. L_1 und L_2, zwei abwechselnd einzuschaltende Lichter. 1 und 2, stark vergrösserte Umrisse zweier Facettenaugen, bei 1 reagiert das Tier nicht, weil trotz der Umschaltung des Lichtes der Lichtstrahl im gleichen Ommatidium bleibt. Bei 2 reagiert es, weil ein neues Ommatidium gereizt wird. (Original.)

Beim Käfer *Chrysomela fastuosa* beträgt der am Auge gemessene Öffnungswinkel β 5·4 Grad. Aus dem Verhältnis α/β ergibt sich also, dass

für $\alpha = 5\cdot1°$ in 94·4 % der Fälle eine positive Reaktion zu erwarten ist
    „ $\alpha = 3\cdot2°$ „ 59·2 %   „   „   „   „
    „ $\alpha = 1\cdot9°$ „ 35·2 %   „   „   „   „
    „ $\alpha = 1\cdot3°$ „ 27 %   „   „   „   „

Die wirklich beobachteten Zahlen sind in der gleichen Reihenfolge 91, 67, 40 und 26 %. Die Übereinstimmung ist also eine sehr gute und die Annahme, dass die

Reaktion nur dann eintritt, wenn der Lichtstrahl von einem Ommatidium auf das nächste überspringt, ist hiermit beweisen. Figur 13 zeigt für eine ganze Reihe von Käfern sowie für die Assel *Porcellio*, dass der Winkel, um den man den Lichtstrahl drehen muss, damit sich in 70 % positive Reaktionen ergeben, proportional dem Ommatidienwinkel ist. Dies ist ein neuer Beweis für die oben aufgestellte These.
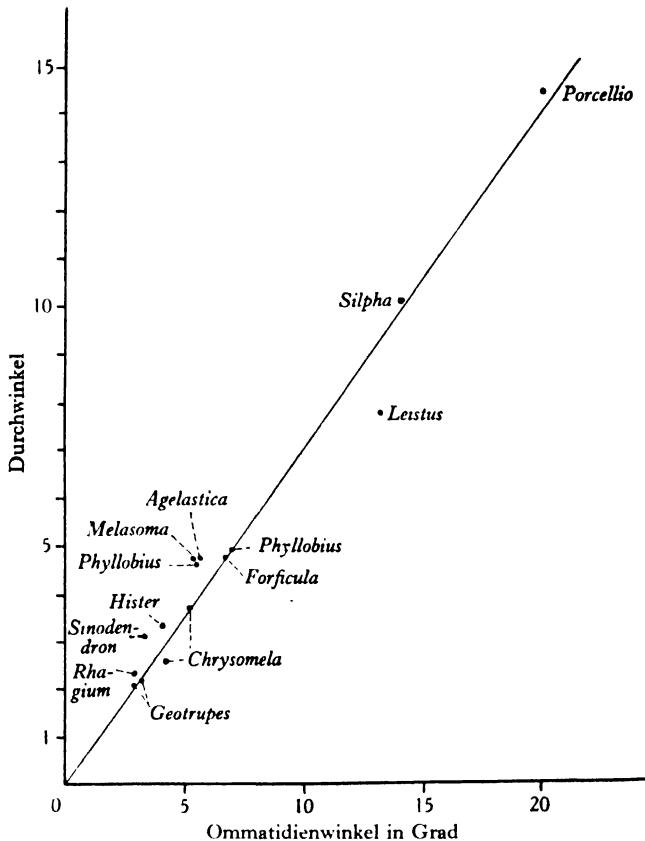
Fig. 13. Beziehung zwischen der Grösse des Ommatidienwinkels und desjenigen Winkels, bei welchem 70 % positive Reaktionen auftreten. (Nach v. Buddenbrock und Schulz, 1933.)

Die Lichtkompassbewegung ist auch für fliegende Insekten von Bedeutung. Wolf (1926–7) konnte in einer schönen Untersuchung für die Biene zeigen, dass sich diese Tiere in einem Gelände, in dem andere optische Merkzeichen fehlen, mit Hilfe der Lichtkompassreaktion orientieren.

Die biologische Bedeutung der Lichtkompassorientierung liegt darin, dass sie dem Tiere ermöglicht, unter der Einwirkung der Sonnenstrahlen oder anderer Lichter geradeauszulaufen. Im Dunkeln vermögen dies die Arthropoden nicht. Die Wirkung der Lichtkompassorientierung zeigt sich besonders deutlich, wenn man ein Tier beobachtet, dem ein Fühler oder ein Vorderbein amputiert ist. Im

Dunkeln beschreibt es fortwährend Kreise, im Hellen läuft es wie ein normales geradeaus. Von besonderer Bedeutung ist die Lichtkompassorientierung für solche Tiere wie die Ameisen, die zwischen Nest und Futterplatz hin- und her-wandern. Brun zeigte schon 1914 dass Ameisen, die man mitten auf ihrem Wege für einige Stunden in eine Schachtel einsperrt, nach ihrer Befreiung nicht mehr den alten Weg zum Nest fortsetzen, sondern um einen Winkel abweichen, um den die Sonnenstrahlen inzwischen ihre Richtung verändert haben.

(g) *Die Empfindlichkeit der verschiedenen Ommatidien.*

Die Ungleichheit der Ommatidien zeigt sich nicht nur in der Verschiedenheit der Reflexe. Für die Fliegen (*Eristalis*) konnte von Dolley und Wierda (1929) nachgewiesen werden, dass die Lichtempfindlichkeit der mittleren Ommatidien etwa 50-mal so gross ist wie die der vorderen, die der hintersten ist wahrscheinlich noch sehr viel bedeutender. Geschlossen wird dies aus dem Verhalten der Tiere im Zweilichterversuch. Setzt man eine Fliege in ein Lichtfeld, das aus zwei sich senk-recht schneidenden Bündeln paralleler Strahlen besteht, so bewegen sich die Fliegen, wenn beide Lichtquellen gleich stark sind, in der Diagonale. Sind die Lichter ungleich, so stellt sich die Fliege mehr in die Richtung des stärkeren ein, ohne aber die Diagonalstellung völlig zu verlassen. Da jetzt keine Drehung, sondern eine geradlinige Vorbewegung eintritt, muss gefolgert werden, dass sich die Reize auf beiden Seiten das Gleichgewicht halten. Das schwache Licht wirkt also auf die seitlichen Ommatidien des rechten Auges ebenso stark ein, wie das starke auf die vorderen Ommatidien des linken. Folglich sind die erstgenannten bedeutend empfindlicher.

(3) DER FORMENSINN.

Der optische Eindruck ruhender Formen führt, soweit wir dies wissen, nur verhältnismässig selten bei den Arthropoden zu bestimmten Bewegungshandlungen. Es darf vielleicht behauptet werden, dass gerade in diesem Punkte ein scharfer Unterschied zwischen den einfacheren und den höher entwickelten Augen besteht. Die Domäne der einfachen Facettenaugen ist das Richtungssehen, die Struktur spielt bei den optischen Reaktionen, z. B. eines Käfers, überhaupt keine Rolle, nur bei den Formen, deren optisch höhere Leistungsfähigkeit sich schon durch die Grösse der Augen dokumentiert: Fliegen, Schmetterlinge, Hymenopteren, ist bisher ein Formensehen, d. h. eine typische Reaktion auf ruhende Formen nach-gewiesen.

Der einfachste Fall bezieht sich auf die Fliegen. Buddenbrock (1935) gibt für die Schlammfliege *Eristalis tenax* den folgenden Versuch an: Das entflügelte Insekt wird auf ein Laufbrett gesetzt, das rechts und links durch eine senkrechte Wand begrenzt ist, am Ende der Bahn befindet sich eine diffuse Lichtquelle, auf welche das phototaktische Tier zuläuft. Die eine Wand wird mit einem einfarbigen Papier bespannt, das weiss, grau oder schwarz sein kann, die andere mit einem gemusterten Papier, z. B. einem schwarz-weiss gestreiften. Die Fliege, die von

einem Ende startet, läuft nun niemals geradeaus, sondern stets schräg oder im Bogen auf die gestreifte Wand zu. Da es gleichgültig ist, welche Helligkeit die andere Wand besitzt, so ist es sicher, dass wir es hier nicht mit einer phototaktischen Reaktion zu tun haben, sondern dass das Tier positiv auf das Streifenmuster reagiert. Diese Reaktion könnte nun zweierlei bedeuten. Es wäre denkbar, dass die Fliege auf die retinalen Verschiebungen anspricht, die notwendigerweise entstehen, wenn sie an den senkrechten Streifen vorbeiläuft, es kann aber auch sein, dass sie auf die ruhende Struktur anspricht. Eine Entscheidung dieser wichtigen Frage ermöglicht die Gegenüberstellung eines senkrechten und eines wagerechten Streifenmusters. Es zeigt sich jetzt, dass die Fliege keine deutliche Entscheidung trifft, obgleich das wagerechte Muster fast gar keine, das senkrechte sehr erhebliche retinale Verschiebungen im Gefolge hat. Folglich kann die retinale Verschiebung nicht der wirksame Reiz sein.

Indem man dem Tiere verschiedene Streifenmuster zur Wahl stellt, kann man prüfen, ob die Fliege ein feiner abgestuftes Formensehen besitzt. Es zeigt sich dann, dass Streifen von einer bestimmten Breite, die also vom Insekt unter einem bestimmten Winkel gesehen werden (etwa 8°–5°), bevorzugt werden. Derartige Versuche haben bewiesen, dass bei Anwendung äquidistanter Streifen, bei denen die Zwischenräume ebenso breit wie die schwarzen Streifen selbst sind, ein System von 20 mm. Breite sowohl breiteren als auch schmaleren vorgezogen wird. Dies bedeutet wahrscheinlich, dass für die Fliege das konturreichere Muster, das also mehr schwarz-weisse Grenzen besitzt, anziehend wirkt. Anderseits wirkt der einzelne schwarze Streifen umso stärker, je breiter er ist. Diese Bedingungen widersprechen einander und so kommt es, dass sich bei 20 mm. das günstigste Kompromiss ergibt. Ausser diesen Faktoren ist auch der Helligkeitsunterschied von Bedeutung, grau-weisse Muster sind weniger wirkungsvoll als schwarz-weisse.

Vergleicht man hiermit den Formensinn der Biene, der vor allem durch die weittragenden Untersuchungen von Hertz (1928–31) aufgeklärt worden ist, so kommt man zu ähnlichen Gesetzmässigkeiten. In Spontanwahlversuchen, in denen das undressierte Tier von zwei ihm gebotenen Mustern das eine bevorzugt, sowie in Dressurversuchen zeigt sich, dass zwei verschiedene Merkmale von ausschlaggebender Bedeutung sind: die Gliederung der Figur oder, wie man auch sagen kann, der Konturreichtum derselben und zweitens die Helligkeitsunterschiede oder Abhebungen, welche die einzelnen Teile der Figur gegeneinander besitzen. Beide Merkmale können sich unter Umständen die Wage halten, so kann ein stark gegliedertes aber schwach abgehobenes (graues) Muster ebenso stark wirken wie ein schwach gegliedertes aber stark abgehobenes (schwarz-weisses) Muster. Die anziehende Wirkung, die häufig von körperlichen Formen ausgeht, beruht auf die in denselben auftretenden Schatten.

Die Wirksamkeit des Konturreichtums ist neuerdings von anderer Seite nachgeprüft worden mit dem Erfolge einer vollen Bestätigung der Hertzschen Resultate. In der Versuchen von Zerrahn (1934) wurden den Bienen, die zu dem Versuchstisch gelockt waren, eine Anzahl von Figuren geboten, die, in der äusseren Form und in der Abhebung übereinstimmend, durch den Konturreichtum (Länge der

schwarz-weissen Grenzlinien) sich unterschieden (Fig. 14). Die Anflugzahlen an die einzelnen Figuren beweisen, dass eine ungefähre Proportionalität besteht zwischen Wahlzahl und Konturlänge.
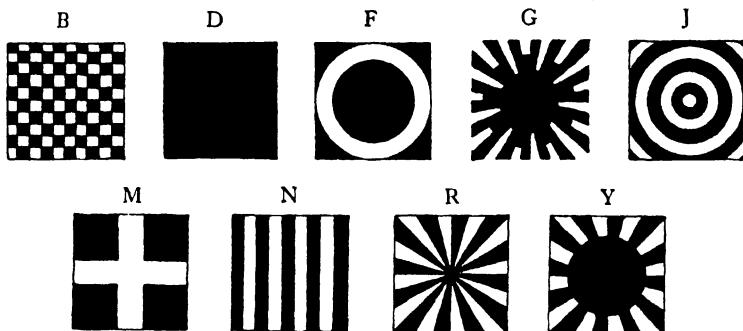
Fig. 14. Spontanwahlserie (ohne Dressur) für Bienen. Näheres im Text. (Nach Zerrahn, 1934.)

Tabelle IV. *Runde Figuren.*

Figuren	Konturlänge cm.	Wahlzahl	Wahlzahl ^o_o
D	31·4	9	4·2
F	53·4	8	3·7
M	54·6	18	8·3
Y	62·7	27	12·5
N	91·6	17	7·9
G	93	27	12·5
J	13	24	11·1
R	121·3	41	19·0
B	16·4	45	20·8

Die einzig mögliche Interpretation dieser Ergebnisse ist wohl, dass für den Reizerfolg die Zahl der gleichzeitig von einem Lichtwechsel betroffenen Ommatidien massgebend ist. Da die Biene während des Anfluges sich hin- und herbewegt, ist die Länge der Schwarzweissgrenzen ausschlaggebend.

Mit diesem Befunde stimmt sehr schön überein, dass Wolf (1933), der in der Dunkelkammer den Bienen flimmernde Felder von verschiedener Flimmerfrequenz bot, fand, dass die anlockende Kraft dieser Felder mit der Flimmerfrequenz wächst. Ebenso gehört hierher, dass Bienen, denen man zwei gleiche Figuren vorsetzt, von denen die eine rotiert, die andere stillsteht, der rotierenden den Vorzug geben (Wolf).

Es würde aber ganz falsch sein, wollte man aus diesen Ergebnissen schliessen, dass der Formensinn der Biene nur auf derartigen sehr einfachen sinnesphysiologischen Vorgängen beruht, bei denen letzten Endes die Summation zahlreicher Einzelimpulse den Ausschlag gibt. Vielmehr hat Hertz (1931) nachgewiesen, dass es im optischen Empfindungsleben der Biene auch höhere sogenannte figurale Merkmale gibt, die mit den bisher aufgedeckten Komponenten, dem Konturreich-

tum und der Abhebung nichts zu tun haben. Als Beispiel sei eine Versuchsgruppe herausgegriffen, bei der den Bienen die Wahl gelassen wird zwischen einem aus zahlreichen grauen Quadraten zusammengesetzten Kreis und einem Ring, der dieselben Elemente aufweist (Fig. 15). Es ist klar, dass die Abhebung in beiden Fällen dieselbe ist, und dass der Konturreichtum pro Flächeneinheit beim Kreise überwiegt. Trotzdem entscheiden sich die Bienen für den Ring, der ihnen als das differenziertere Gebilde erscheint.
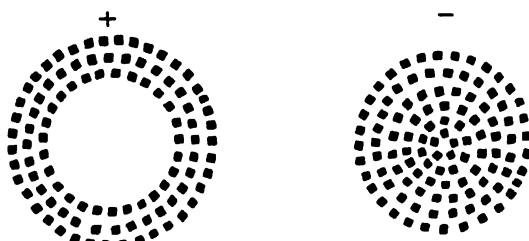
Fig. 15. Biene, Wahlversuch zwischen Ring- und Kreisgruppe. Die Quadrate sind im Original grau statt schwarz. (Nach Hertz, 1931.)

Für die Tagfalter ist früher verschiedentlich behauptet worden, dass sie imstande wären, ihre Weibchen durch den Gesichtssinn zu finden und zwar umso leichter, je täuschender für das menschliche Auge das vorgesetzte Modell dem wirklichen Weibchen wäre. Nach neueren Versuchen von Ilse (1932) muss dies als zweifelhaft erscheinen. Ilse fand bei *Argynnis paphia*, dass von weiblichen Faltern, Modellen sowie lebenden Tieren unter Petrischalen, keine andere Wirkung ausging, als die Auslösung von Nahrungsreaktionen (Ausstrecken des Rüssels). Diese Reaktion wird von jeder farbigen Fläche ausgelöst, jedoch lässt sich zeigen, dass bei einer Wahl die Grösse der Fläche und ihr Konturreichtum von Ausschlag sind. Es kommt also auch hier ersichtlich auf die Zahl der zugleich gereizten Ommatidien an.

Dass gewisse Insekten auf ruhende Formen reagieren, ergibt sich in manchen Fällen auch ohne Experiment aus der einfachen Beobachtung. Das wichtigste Beispiel dieser Art ist die Ortsorientierung der socialen Hymenopteren, vor allem der Biene, von der seit langem bekannt ist, dass sie die Umgebung ihres Stockes im Umkreise von mehreren Kilometern kennt. Dies setzt voraus, dass sich das Insekt die Landschaft, die ihr als ein Nebeneinander verschieden farbiger und verschieden heller Flächen erscheinen muss, bei den Orientierungsflügen optisch einprägt.

Die übrigen Beobachtungen beziehen sich auf sehr viel einfachere Dinge. So gibt es eine alte Beobachtung von Forel (1910), dass Wespen in die Wand geschlagene Nägel anflogen, vermutlich sie mit Fliegen verwechselnd, auf welche sie Jagd machten. Knoll (1922) beobachtete, dass der Taubenschwanz (*Macroglossa stellatarum*) auf der Suche nach Winterquartieren schwarze Flächen anfliegt. Dies könnte an sich als positive Skototaxis gedeutet werden, aber der Falter bevorzugt deutlich Flächen von bestimmter Grösse, zeigt also wohl gewisse Anfänge eines Formensinns.

(4) Der Farbensinn.

Der Farbensinn der Arthropoden, insbesondere der Insekten, ist in den letzten Jahren ziemlich sorgfältig studiert worden, sodass sich ein einigermassen abgerundetes Bild von ihm entwerfen lässt. Der Farbensinn ist durch den ganzen Tierstamm hindurch zu verfolgen, selbst bei sehr niedrig stehenden Formen, wie den Cladoceren (*Daphnia*), ist er überzeugend nachgewiesen worden. Anderseits fehlt es aber auch nicht an farbenblinden Arten. Während es aber bei den Wirbeltieren sehr leicht ist, biologische Beziehungen zu erkennen, indem nächtlich lebende Tiere farbenblind, Tagtiere dagegen farbensehend zu sein pflegen, gibt es hierfür bei den Arthropoden keine Parallele. Es gibt nicht nur Schmetterlinge (Sphingiden) die in der Dämmerung, in der der Mensch längst zum Stäbchensehen übergegangen ist, noch Farben sehen, auch die typischen Nachteulen, deren eigentliche Flugzeit die finstere Nacht ist, zeigen im Experiment eine deutliche Fähigkeit, Farben zu unterscheiden (Schlegtendal, 1934). Anderseits kennen wir unter den anscheinend farbenblinden sowohl Taginsekten wie gewisse Wanzen, als auch Nachtinsekten wie *Dixippus morosus*.

Gerade für die Arthropoden sind eine ganze Reihe verschiedener Methoden erdacht worden, um den Farbensinn nachzuweisen. Die bekannteste ist die Dressurmethode, die ja auch meistenteils für Wirbeltiere angewandt wird. Sie besteht darin, dass eine Association hergestellt wird zwischen einem Futterreiz und einer gleichzeitig mit dem Futter dargebotenen Farbe. Es lässt sich dann meist zeigen, dass das Tier nach einiger Zeit auf die Dressurfarbe allein—auch ohne Futter—anspricht, andere Farben dagegen unberücksichtigt lässt. Mit dieser Methode ist es vor allem gelungen, den Farbensinn der Biene weitgehend aufzuklären (v. Frisch, Kühn, u. a.), ferner ist der Farbensinn der Libellenlarven durch Dressur ermittelt worden (Koehler, 1924). Eine angeborene "Naturdressur" zeigen viele auf den Besuch bunter Blüten eingestellte Taginsekten. Knoll (1922) konnte zeigen, dass der Taubenschwanz (*Macroglossa stellatarum*) sowie die Fliege *Bombylius fuliginosus* Blau von jedem Grau unterscheidet. Ilse (1928) konnte den Nachweis führen, dass frischgeschlüpfte Tagfalter sofort farbigen Kunstblumen den Vorzug vor gleichgestalteten und gleichgrossen grauen geben.

Indessen versagt diese Methode bei sehr vielen psychisch tiefstehenden Arthropoden; man ist daher bei ihnen gezwungen, andere Wege einzuschlagen. Manche dieser Methoden haben nur speciellen Charakter. So kann man bei Krebsen mit bunten Chromatophoren das Vorhandensein eines Farbensinnes daran erkennen, dass jede Chromatophorenart auf dem ihr gleichgefärbten Untergrunde besonders stark expandiert wird, die roten auf rotem Grunde, die gelben auf gelbem (Koller, 1927, bei *Crangon*). Die Bevorzugung grauer Schneckengehäuse vor farbigen beliebiger Helligkeit zeigt, dass die Einsiedlerkrebse blau und gelb aber nicht rot und grün von grau unterscheiden können (Koller, 1928).

Man hat ferner versucht die Phototaxis, die Lichtkompassorientierung und die optomotorischen Reaktionen zum Nachweise des Farbensinnes heranzuziehen.

Bei der optomotorischen Methode (Buddenbrock u. Friedrich, 1933) handelt es

sich wie bei der Dressur darum, nachzuweisen, dass zwei Farben trotz gleicher Hellig-
keit nicht verwechselt werden. Zunächst ist eine Gleichung herzustellen zwischen
einer bestimmten Farbe und einem Grau derart, dass das Tier auf die Drehung
einer Trommel, die abwechselnd diese beiden Streifen aufweist, nicht anspricht. Es
ist also Blau$_x$ = Grau$_y$. In einer zweiten Versuchsserie wird jetzt zu Grau$_y$ ein pas-
sendes Gelb$_z$ gesucht. Das Tier reagiert also auch auf die Kombination Grau$_y$ Gelb$_z$
negativ. Wenn nur die Helligkeiten von Ausschlag sind, muss dem Tiere jetzt auch
Gelb$_z$ und Blau$_x$ gleich erscheinen, es darf also nicht auf die Drehung einer Trommel
ansprechen, die aus abwechselnden Streifen dieser beiden Farben besteht. Der
Versuch zeigt aber, dass eine Reaktion sehr wohl eintritt. Folglich hat das Tier die
Fähigkeit, Gelb von Blau zu unterscheiden. Mit dieser Methode ist es gelungen,
den Farbensinn verschiedener Käfer, gewisser Nachtschmetterlinge und einiger
Krebse nachzuweisen (Schlegtendahl, 1934). Aber auch sie ist nicht allgemein
verwendbar, da es zahlreiche Arthropoden gibt, die überhaupt keine optomotor-
ischen Reaktionen zeigen.

Die Phototaxis verwendete Hamilton (1922), in der folgenden Art, zum Nachweis
des Farbensinns: Die Versuchstiere, Fliegen, werden in ein Glasrohr getan, das von
beiden entgegengesetzten Seiten stark beleuchtet wird, links farbig, rechts weiss.
Man verschiebt die beiden Lichter solange, bis sie den Tieren gleich hell er-
scheinen, was man an ihrer gleichmässigen Verteilung erkennt. Sie werden jetzt
längere Zeit nur durch das farbige Licht beschienen. Knipst man hierauf das
weisse Licht wieder an, so gehen sie alle zum Weiss, da für das farbige Licht in-
zwischen eine Ermüdung eingetreten ist. Aus dieser speciellen Ermüdbarkeit für
einzelne Farben wird geschlossen, dass die Versuchstiere die Farben von Weiss
unterscheiden.

Die Beziehung zwischen Farbensinn und Lichtkompassbewegung ist noch
nicht völlig spruchreif und sei daher hier übergangen.

Der Farbensinn der Arthropoden scheint in viel stärkerem Masse, als wir dies
von den Wirbeltieren wissen, den besonderen biologischen Bedingungen angepasst,
also specialisiert zu sein. So ist es sehr auffallend, dass blütenbesuchende Insekten
Grün, das für sie keine biologische Bedeutung hat, nur schwer oder garnicht von
Grau unterscheiden. Blattfressende Käfer dagegen haben für Grün ein sehr aus-
gesprochenes Farbempfinden. Hierhin gehört auch, dass der Krebs *Leander
adspersus*, der vorwiegend rote Chromatophoren besitzt, im optomotorischen
Versuch hauptsächlich diese Farbe, nicht aber Gelb und Blau, von Grau unter-
scheidet (Schlegtendal, 1934).

Hinsichtlich seiner Differenzierung ist der Farbensinn der Arthropoden auf
einer viel tieferen Stufe stehen geblieben als derjenige der Wirbeltiere. Im primi-
tivsten Falle wird überhaupt nur zwischen kurzwelligem und langwelligem Licht
unterschieden (*Daphnia*, v. Frisch u. Kupelwieser, 1913). Selbst die Biene, die von
allen Insekten wahrscheinlich den höchst enwickelten Farbensinn besitzt, bringt es
nach den sehr exakten Untersuchungen von Kühn (1927), nur zur Unterscheidung
von vier Spektralbezirken: 650–530$\mu\mu$ (Rot, Gelb, Grün); 510–480$\mu\mu$ (Blaugrün);
470–400$\mu\mu$ (Blau und Violett); 400–$ca.$ 300$\mu\mu$ (Ultraviolett). Innerhalb dieser vier

Hauptbezirke wird der jeweils hellste Bezirk bevorzugt. Bietet man z. B. Bienen, die auf Rot von 620–650μμ dressiert sind, ein kontinuierliches Spektrum an, so sammeln sie sich im Gelb und Grün, das ihnen qualitativ gleich dem Rot erscheint, aber bedeutend heller ist.

Es ist kein einziges Insekt bekannt, dass über dieses Unterscheidungsvermögen von vier Spektralbezirken hinausgeht. So ist von den Schwärmern (Sphingiden) vorläufig nur erwiesen, dass sie zwei Qualitätsbezirke unterscheiden (Orange, Gelb, Grün—Blau, Violett). Bei den Tagfaltern ergibt die Zählung der Anflüge zu verschieden gefärbten Papierblumen bei einigen Arten nur zwei Bevorzugungsgipfel, bei anderen deren vier (Ilse, 1928).

Besonders interessant ist, dass sich gewisse Eigentümlichkeiten des Farbensehens, die sonst nur bei den Wirbeltieren sich finden, auch bei den Bienen nachzuweisen sind. So konnte Kühn (1921) durch einen geistvollen Kunstgriff den simultanen Farbenkontrast der Biene nachweisen. Blau dressierten Bienen werden graue Papierringe vorgesetzt, die auf verschiedenen Unterlagen: Weiss, Grau, Schwarz und Gelb verteilt werden. Die Bienen sammeln sich nur auf denjenigen Ringen, die auf Gelb liegen. Dies kann nur so verstanden werden, dass das Grau auf dem Gelb dem Bienenauge den Eindruck von Blau erweckt.

Der Bereich des sichtbaren Spektrums ist bei den Arthropoden häufig etwas anders als beim Menschen. Vor allem ist auffallend, dass eine Anzahl systematisch weit auseinanderstehender Arthropoden Ultraviolett als gesonderte Farbe wahrnehmen. Bewiesen ist dies für die Biene (Kühn, 1927), für verschiedene Nachtschmetterlinge (Merker, 1929), für die Stabheuschrecke *Dixippus morosus*, sowie für den Wasserfloh (*Daphnia*), wahrscheinlich gilt es auch für die Raupe des Weisslings (*Pieris*, nach Brecher, 1917) und viele andere Formen. Die Empfindlichkeit für Ultraviolett geht bei der Biene bis etwa 310μμ, bei den Schmetterlingen bis 240μμ, bei *Daphnia* sollen sogar Strahlen noch wirksam sein, die im Sonnenlicht garnicht mehr vorhanden sind.

Im Zusammenhang hiermit steht vielleicht die Tatsache, dass auf der langwelligen Seite das Spektrum bei einem Vergleich mit dem unseren vielfach verkürzt erscheint. Die Biene verwechselt Rot mit Schwarz. Wie die beistehende Tabelle zeigt, gilt dies aber keineswegs allgemein. In dieser Tabelle ist angegeben, mit welchem Grau im optomotorischen Drehversuch das untersuchte Tier eine bestimmte Farbe verwechselt. *Hesperia comma* verwechselt also Rot 2 mit einem Grau, das nur 6 Weissprocente enthält, also beinahe schwarz ist. *Aporia crataegi* sieht Rot 2 ebenso hell wie ein Grau mit 33 % Weiss.

<div align="center">Tabelle V (nach Schlieper, 1928).</div>

Tierart	Rot 2	Blau 12	Gelb 4	Grün 7
Hesperia comma	6	26	42	64
Apis mellifica	11	22	57	61
Vanessa urticae	13	22	61	69
Papilio machaon	16	25	57	64
Pieris brassicae	24	16	69	60
Aporia crataegi	33	22	79	74
Mensch dunkeladapt.	9	26	42	64
Mensch helladapt.	31	22	79	74

Das Helligkeitsmaximum des Insektenauges scheint nach den Feststellungen verschiedener Autoren (v. Hess, Mast, Sander) bei *ca.* 530 mμ zu liegen.

III. ZUSAMMENFASSUNG.

In der vorliegenden Arbeit wird eine zusammenfassende Darstellung unserer jetzigen Kenntnisse von der physiologischen Leistung des Facettenauges gegeben.

Im ersten Teil wird das einzelne Ommatidium betrachtet und die Beweise dafür gebracht, dass das Ommatidium wirklich die physiologische Einheit des Facettenauges ist. Als Leistungen des einzelnen Ommatidiums werden in diesem Abschnitt das Unterscheidungsvermögen und die Adaptation des Facettenauges besprochen.

Im zweiten Teil wird die Leistung des gesamten Auges untersucht und zunächst gezeigt, dass sich eine Anzahl verschiedener Reflexe unterscheiden lassen: die Phototaxis, die Reaktionen auf Bewegung, die tonischen Reflexe, der sogenannte Lichtrückenreflex, die Lichtkompassorientierung. Zum Teil ergibt sich, dass den einzelnen Augenteilen ganz bestimmte Reflexe zugeordnet sind.

Auch der im nächsten Abschnitt behandelte Formensinn trägt zum Teil noch den Charakter einfacher reflektorischer Handlungen, zeigt aber, besonders bei der Biene, bereits das Wesen eines höheren psychischen Geschehens.

Im letzten Kapitel wird der im Arthropodenstamme weit verbreitete Farbensinn behandelt: die Methoden seiner Erforschung, das Unterscheidungsvermögen für verschiedene Farben, die Begrenzung des sichtbaren Spektrabezirks.

Auf Grund der bekannt gewordenen Tatsachen kann zu der Frage Stellung genommen werden, ob innerhalb des ganzen Arthropodenstammes eine bestimmte fortschrittliche Tendenz des Facettenauges zu beobachten ist. Vom Standpunkte des Systematikers ist diese Frage zu verneinen. Im Gegensatz zu den Wirbeltieren kann man bei den Arthropoden keine Linienführung feststellen, die sich mit der Reihe Fisch, Amphibium, Sauropsid, Säugetier vergleichen liesse. Die Weiterentwicklung des Facettenauges von einem niederen zum höheren Typ ist in den verschiedensten Gruppen zu beobachten und trägt grösstenteils adaptiven Charakter.

Das niedere Facettenauge ist vom höheren unterschieden durch geringere Ommatidienzahl, grösseren Winkelraum und geringere Länge des Ommatidiums. Von den Funktionen des niederen Auges lässt sich aussagen, dass hier das Richtungssehen das beherrschende Princip ist. Es ist zweifelhaft, ob bei irgend einem Tier, das zu dieser Gruppe gehört, die Fähigkeiten über die Phototaxis und die Lichtkompassorientierung hinausgehen. Anderseits sind diese Fähigkeiten auch bei den am höchsten entwickelten Augen: Biene, Fliege, etc. zu beobachten, sie lassen sich also durch den ganzen Arthropodenstamm hindurch verfolgen. Ähnlich liegen die Dinge beim Farbensinn, der gleichfalls nicht etwa das Vorrecht der besser entwickelten Augen ist, sondern allerorts sich findet. Wo er fehlt, scheinen besondere biologische Verhältnisse vorzuliegen.

Das Bewegungssehen, insbesondere die jetzt so viel untersuchten optomotorischen Reaktionen, scheinen nach den bisherigen Befunden den niederen Augen zu fehlen. Es ist wenigstens vergeblich versucht worden, sie bei Isopoden, *Forficula*

u. a. niederen Formen nachzuweisen. Anderseits fehlt aber diese eigentümliche Reaktionsart auch vielen anderen Tieren, z. B. gewissen Schmetterlingen (Geometriden, Bombyciden) ohne dass wir hierfür einen Grund wüssten.

Der schärfste Unterschied zwischen höheren und niederen Facettenaugen scheint auf dem Gebiete des Formensehens zu liegen. Bei den Versuchen über den Formensinn von *Eristalis* (vgl. S. 306) ergab es sich jedenfalls, dass bei Käfern und anderen mit geringer entwickelten Augen versehenen Formen keine Reaktion zu erzielen war, und auch sonst ist nichts von einem noch so primitiven Formensinn dieser Tiere bekannt.

IV. SUMMARY AND CONCLUSIONS.

In the present review an account has been given of the state of our knowledge concerning the mode of action of the compound eye.

The single ommatidium is first considered, and proof is furnished that this is the real physiological unit of the eye. Resolution and adaptation are dealt with as functions of the individual ommatidium.

In relation to the action of the eye as a whole, it is necessary to distinguish a number of different reflexes, such as phototaxis, reactions to movement, tonic reflexes, the so-called dorsal light reflex, and light-compass-orientation. Certain of these reflexes are connected with definite parts of the eye.

The sense of form has also, in part at least, the character of simple reflex behaviour, but it shows in addition, particularly in the bee, a higher psychic nature.

The sense of colour, so widely spread among arthropods, is discussed from the points of view of experimental procedure, ability to distinguish colours, and limits of the visible spectrum.

An answer can be given, on the basis of known facts, to the question as to whether or not there exists a progressive tendency in the compound eye throughout the arthropod phylum. From the systematic standpoint the answer is in the negative. No linear progress is traceable in arthropods comparable with that in the series fish, amphibian, sauropsid, mammal. The progressive development of the compound eye from a lower to a higher type is found in the most diverse groups of arthropods and is principally of an adaptive character.

The lower compound eye is distinguished from the higher by having fewer and shorter ommatidia, and a wider angle. Concerning the mode of action of the lower eye it may be said that its most important function is direction-sight. It is doubtful whether the capacities of any animal possessing eyes of this type extend beyond phototaxis and light-compass-orientation. These reactions, however, exist also in animals with the most highly developed eyes (bees, flies, etc.); they are found throughout the arthropods. This is also true of the sense of colour, which likewise is not the prerogative of the most highly developed eyes but is found in all sorts of arthropods. When a colour-sense is lacking, special biological circumstances seem to be involved.

Movement-sight, particularly the optomotor reactions which have been so extensively studied, appears to be absent in the case of lower eyes. At all events it has been sought in vain in the isopod *Forficula* and in other lower forms. But this peculiar type of reaction is also absent, for unknown reasons, in numerous other animals, for example in certain Lepidoptera (Geometridae, Bombycidae).

Form-sight seems to provide the sharpest distinction between higher and lower compound eyes. In the course of experiments on the form-sight of *Eristalis* it transpired that in beetles and other animals with less well-developed eyes no reaction could be obtained. Nor is there other evidence of any primitive form-sight in such animals.

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Seit der Niederschrift der Arbeit sind eine Reihe neuer Untersuchungen veröffentlicht worden, die nicht mehr berücksichtigt werden konnten.

THE EVAPORATION OF WATER FROM INSECTS

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CONTENTS.

I. INTRODUCTION.

In recent years, the importance of the relations between insects and climatic factors has been increasingly realised, and a great deal of work has been done on various aspects of the subject. One important problem which has received much attention is that of the factors influencing the loss of water from insects. For accounts of the earlier work, reference should be made to the summaries of Uvarov (1931) and Buxton (1932). Since they were written, recent work has made it possible to re-interpret many of the apparently discordant results and fit them into a simple scheme. We are now able to state more precisely how both external conditions and insect metabolism affect the rate at which water is evaporated. Only work connected directly with evaporation will be considered within the scope of the present paper, and so little has been done on the effects of moving air that we must consider results obtained in still air only. Air currents probably have less effect on insects than on other groups of animals, as so many species are found in situations where the air must normally be still.

It will perhaps be worth while at this point to try and make clear the relationship between the various measures of atmospheric humidity in common use. At any temperature, air in equilibrium with an excess of water ("saturated air") contains a definite amount of water vapour. This is usually expressed as the *saturation vapour*

pressure, in millimetres of mercury. The higher the temperature the higher is the saturation vapour pressure. The amount of water vapour present in any sample of air can be expressed as the vapour pressure, in millimetres of mercury; this is called the *absolute humidity*. If the absolute humidity is expressed as a percentage of the saturation vapour pressure at the same temperature, the result is the *relative humidity*. As the saturation vapour pressure rises with rising temperature, so the absolute humidity for any given relative humidity rises also. The *saturation deficiency* of the air, which can be said to measure its "dryness," is the difference between the absolute humidity and the saturation vapour pressure at the same temperature. It is expressed in millimetres of mercury. Table I may help to make this subject clearer. It shows how the moisture in samples of air with a relative humidity of 20 per cent. at temperatures of 10°, 20° and 30° C. may be expressed in terms of absolute humidity and saturation deficiency[1].

<div align="center">Table I.</div>

Temperature ° C.	Saturation vapour pressure mm.	Absolute humidity of air with R.H. of 20 % mm.	Saturation deficiency of air with R.H. of 20 % mm.
10	9·2	1·8	7·4
20	17·5	3·5	14·0
30	31·7	6·3	25·4

The fact that the rate of evaporation of water from a free surface is approximately proportional to the saturation deficiency of the air is one implication of Dalton's law. Bacot and Martin (1924) and Gunn (1933) found that evaporation took place from surfaces of water, or from "artificial insects" made of damp filter paper, at a rate proportional to the saturation deficiency under similar conditions to those to which they exposed insects. This article shows the way in which water loss from insects resembles and differs from evaporation from inanimate surfaces of water.

<div align="center">II. THE SITE OF LOSS OF WATER.</div>

There are three possible ways in which water might be evaporated from an insect's body, excluding excretion: (1) through the general surface of the body wall; (2) from the tracheal system; and (3) partly from the body surface, and partly from the tracheal system. The fact that carbon dioxide passes readily through chitin (Dewitz, 1890) and that insects get rid of some of that gas through their integument (Buddenbrock and Rohr, 1923) suggests that water vapour may pass from the insect's body in a similar manner. Many insects are able to close their spiracles, while others possess no such mechanism. Hazelhoff (1927) states that the reason why certain resting insects keep their spiracles closed most of the time, only opening

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[1] The excellent graph published by Buxton (1930) should be referred to by anyone who finds it difficult to understand the meaning of the terms used to describe atmospheric humidity.

them sufficiently to obtain enough oxygen, is in order to conserve water. He believes that most of the water evaporated is lost through the spiracles. The work of Gunn (1933) supports this view. He found that with air of the same dryness (same saturation deficiency), the rate at which water was lost by cockroaches (*Blatta orientalis*) increased gradually as the temperature rose from 20° to 30° C. The rate of respiration increased in a parallel manner.

More direct evidence is given by the work of Mellanby (1934 b), who measured the evaporation from insects over periods of only a few hours. The rate of loss of water from various insects was found, first in dry air, and then in mixtures of gases (such as air containing 5 per cent. of carbon dioxide) which cause insects to keep their spiracles open permanently. In insects with mechanisms for closing their spiracles, the rate of loss of water under the second conditions was two to seven
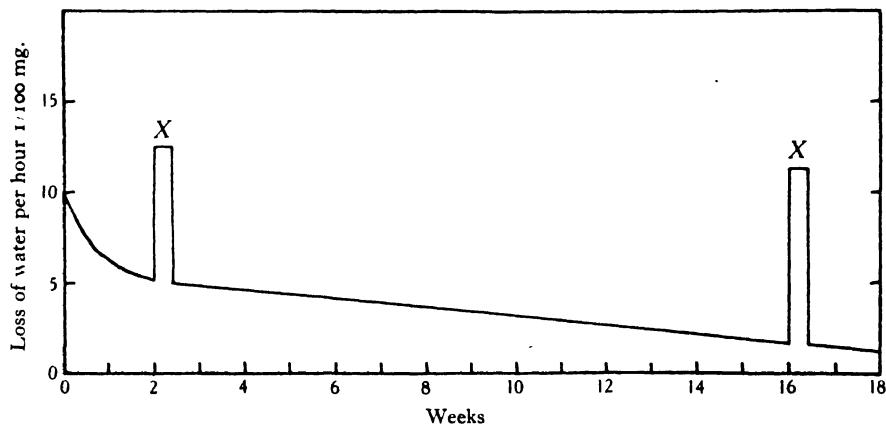
Fig. 1. The rate of loss of water from a fasting mealworm over a period of 4 months. At the points marked *X*, the insects were made to keep their spiracles open. (Mellanby 1934 b, with acknowledgements to the Royal Society of London.)

times that in dry air. In insects which could not close their spiracles, the rate of loss of water was practically the same under all conditions. Fig. 1 shows results obtained using a single mealworm. As starvation (in dry air) proceeded, the rate of loss of water decreased very considerably, so that after 4 months it had fallen to less than a third of the rate after 2 weeks. This decrease was shown to be due to a decrease in metabolism, causing the insect to open its spiracles less frequently. The results obtained when the spiracles were opened showed that it was not caused by any internal "drying up," which slowed down evaporation. With the spiracles permanently open (*X*), the rate of loss of water was not affected by desiccation—it was as great as ever even after 4 months' starvation. Further experiments (Mellanby, 1934 b) support the view that the integument is nearly water-tight, even in the case of insects possessing thin cuticles, such as the flea larva and the clothes-moth larva. The water evaporated from their bodies is lost mainly through their tracheal system. Many insects are susceptible to desiccation, not so much because they lose water by

evaporation, but because they are unable to dry their faeces in the rectum and waste a great deal of water in excretion (Wigglesworth, 1932).

The experiments described above were all done at temperatures of 30° C. and below. Gunn (1933) has shown that the cockroach behaves in a different manner at higher temperatures. Although the saturation deficiency is the same in each case, the rate of loss of water at 35° C. is three to four times as great as the rate of loss at 30° C. Ramsay (1935) has shown that, at temperatures above 30° C., the body surface of the cockroach becomes more permeable to water, due to a phase change, probably melting, of a layer of some fatty or waxy substance which occurs naturally on the surface of the insect. In the light of Ramsay's work, care must be taken in interpreting experiments on other insects at high temperatures, although similar changes in the permeability of the body wall do not occur in all insects. The body wall of the mealworm, for instance (Mellanby, 1932 b), does not appear to be any less impermeable at 37° C. than it does at 30° C.

Evaporation from insects' eggs is governed by different factors. The chorion which covers the surface helps to conserve water, and the permeability of the surface of the egg depends on the stage of its development and the temperature of the environment (Evans, 1934). These changes in surface permeability give rise to results which are analogous to those obtained from larval and adult insects when the spiracles are open to different extents. As Buxton (1932) has pointed out, evaporation from insects' eggs is really even more complicated a problem than evaporation from other stages. If eggs are exposed to different climatic conditions, results will be more easily comparable if the experiments are arranged so that similar morphological changes occur in each case. The simplest way to ensure this is to compare the total development from the laying of the egg to its hatching in each experiment, and from such experiments (Maercks, 1933 a) conclusions regarding the rate of loss of water have been drawn.

III. INTERNAL FACTORS INFLUENCING THE RATE OF WATER LOSS.

Robinson (1928) has shown that there is some relation between the percentage of water in an insect's food, and the percentage of water in its body. He also finds that those insects containing a low percentage of water in their bodies have a high percentage of "bound water" (i.e. much of the water does not freeze when exposed to a temperature of $-20°$ C.). Now these insects are usually able to withstand desiccation, and Robinson assumes that a low percentage of total water, much of which is in the "bound" state, causes the rate of evaporation from these insects' bodies to be slow. At first sight this hypothesis appears reasonable, and has received considerable support. Actually, however, it is difficult to see how either (1) the percentage of dry matter in an insect's body, or (2) the ratio of bound to free water, can affect the rate at which water is lost by evaporation. Obviously, the amount of (free?) water present governs the amount of water which can be lost, but we are at present dealing with the rate of loss of water. If two similar beakers are taken, and one is filled with water and the other is half-filled with sand and then filled up to

the top with water, until the second beaker is almost dry the rate of evaporation will be the same in each case. The surface area, together with the dryness of the atmosphere, governs the rate of loss of water; the total amount of water present has no effect. It is the same with insects. As l ng as there is any water available for evaporation, the rate at which that water is lost ill be unaffected whether there is much or little solid matter or much or little bounu water.

As the proportion of dry matter to liqu'd rises in some insects when they are exposed to dry conditions, it has been assumed that the increase in concentration of their body fluids will slow down evaporation. It is improbable that concentration takes place to a sufficiently marked extent. A solu'ion with a vapour pressure sufficiently low to make evaporation from it much (*i.e.* say 5 per cent.) slower than evaporation from pure water would have to be stronger than any fluid found in an insect's body. Even 10 per cent. saline, which kills ordinary protoplasm on contact, has a vapour pressure only 5 per cent. lower than that of distilled water.

If bound water has a lower vapour pressure than free, and if during desiccation *all* the free water were evaporated first, then the remaining (bound) water might be evaporated only with difficulty. There is, however, no evidence that any insects exist which contain bound water *only*, even after considerable desiccation. As long as an insect's body contains some free water, the fact that it contains some other water which is bound cannot affect the rate at which the free water is evaporated. The presence of a high proportion of bound water appears to be of value to an insect which has to withstand low temperatures, but it cannot help it to withstand dry conditions.

We have other good reasons for assuming that the internal conditions of insects do not affect the rate at which water is evaporated. As has been shown above, it appears that practically all the water lost by evaporation is lost from the tracheal system. Now the work of Wigglesworth (1930, 1931) suggests that the tonicity of the fluid in the tracheoles does not greatly alter during starvation (and desiccation). If the condition of the body fluids does not affect the tonicity of the fluid in the tracheoles, then it cannot affect the vapour pressure of the tracheal air, and the limiting factor which finally governs water loss must be the extent of opening of the spiracles. As insects which withstand desiccation have efficient closing mechanisms, and as they open their spiracles less and less as desiccation proceeds (see p. 319 above), all the experimental facts are explicable without recourse to any hypothesis about bound and free water[1]. It appears to be simply a coincidence that many, insects which withstand desiccation contain a low proportion of water and that much of that water is bound. They withstand desiccation because they close their spiracles, have an impermeable cuticle, and do not excrete water wastefully.

[1] In the case of insect eggs, analogous results may be explained by a change in the permeability of their surface.

IV. THE LAWS GOVERNING THE 'tATE OF WATER LOSS.

(1) THEORETICAL CC,ISIDERATIONS.

The rate of evaporation of water from ' ,iy system in still air is governed by two principal factors: (1) the area of the surfa.e from which evaporation takes place, and (2) the difference between the vapour p essure at the surface and the vapour pressure of the air. When this principle is applied to insects, it appears that the extent to which the spiracles are kept ope,i may be considered as representing the "surface area," and the difference be.ween the vapour pressure of the air in the tracheal system and outside it represents the second factor which influences evaporation.

In many insects, the extent to which the spiracles are opened appears to depend \upon the rate of metabolism, which in turn is mainly governed by the temperature. Therefore, at one temperature and several humidities the spiracles will be opened to approximately the same extent, and the rate of evaporation will be governed simply by the difference between the vapour pressure inside the tracheae and outside. In those insects which possess no mechanism for closing their spiracles, the rate of loss of water should depend solely upon the difference in vapour pressure, and should be independent of temperature.

Now we know that the fluid in the tracheoles—even after the insect has suffered considerable desiccation—must have a vapour pressure nearly equal to that of distilled water. Therefore, when the spiracles are closed, the air in the tracheal system must be nearly saturated. As the body temperature of insects is normally approximately the same as that of the surrounding air, at the moment the spiracles are opened the difference in vapour pressure between the tracheal and the outside air will be equal to the saturation deficiency. In other words, water will evaporate from the tracheal system at a rate governed by the saturation deficiency of the outside air. Whether the evaporation from the insect continues at that rate depends on whether the air in the tracheae remains saturated with water vapour. Experimental evidence goes to show that the air in the tracheae may sometimes become comparatively dry, and so limit the amount of water which can be evaporated (Buxton, 1930, etc.). Why this happens is not at all certain—it is not because the proportion of water in the insect's body has been lowered. Two possible reasons may be suggested. The first is that the limiting factor is the rate at which water vapour can diffuse from the wet tracheoles into the tracheal trunks. When the saturation deficiency is high, it may be that water vapour cannot sufficiently quickly replace that which is lost. The second suggestion is that water vapour enters the tracheae not only from the fluid in the tracheoles but also through the tracheal walls. If these walls possess a property in common with the skin of the newt (Gray, 1928), isolated muscle or even the surface of a gelatine gel, then the results obtained are explicable. A newt loses water from its skin very readily, but the rate of loss of water falls off as time goes on. In water or in a saturated atmosphere, the skin recovers, and on further exposure water is lost for a further period at approximately the original rate. It is not making a very great assumption to suggest

that the lining of the tracheae may have this same property, which appears to be so commonly found in animals. After a certain amount of water has been lost, the tracheal walls may become dry, and all further water vapour lost will have to diffuse from the tracheoles. On closing the spiracles the air inside will become saturated, and then the walls should recover, in the same way that a newt's skin recovers when immersed in water or exposed to saturated air.

The hypothesis that the rate of evaporation of water from insects is usually governed by the saturation deficiency of the air (Buxton, 1931, 1932) appears to be reasonable, and to fit in with established physical laws. Nevertheless, this hypothesis has been questioned by a number of workers (Janisch and Ghabn, 1933; Maercks, 1933 a, b), who state that water loss is frequently governed by relative humidity. On physical grounds it is difficult to see how this could happen. The absolute amount of water which is taken up from the air by a hygroscopic substance depends on the relative humidity, and this fact has caused some workers to expect insects to behave in what they consider to be an analogous manner. But the *rate* at which hygroscopic substances take up water from the air depends, not on relative humidity, but on saturation deficiency. At one relative humidity and several temperatures, although the final amount of water in the substance would be the same in each case, the time taken to reach equilibrium would depend on the saturation deficiency. Insects are unlike inanimate hygroscopic substances in that, by producing water of metabolism in their bodies, they prevent themselves from coming into equilibrium with the surrounding air. Also the proportion of water in a living insect can only vary between narrow limits. No doubt the quantities of water contained in dead bodies of insects are governed by the relative humidity of the air—but even dead bodies will gain or lose water at a *rate* governed by saturation deficiency. Some of the figures given by Maercks (1933 a), and said by him to support the view that the rate at which insect eggs lose water is proportional to the relative humidity, are given on p. 328, and it will be seen that in reality they support the view that water loss is governed by saturation deficiency.

Whenever the internal temperature of an insect's body is different from that of the surrounding air, the evaporation of water will be affected. Insects' body temperatures may be raised by the absorption of radiation (Buxton, 1924) or by the effects of muscular energy (Bachmetjew, 1901), and cooled by the evaporation of water (Necheles, 1924). As a general rule, however, the bodies of insects are so small that they cannot maintain a temperature different from their surroundings for long periods (see Mellanby, 1932 a). The only condition under which the slight differences in temperature between the insect's body and the air would affect evaporation to any great extent would be in very moist air. Here any difference between the insect's temperature and atmospheric temperature would increase the difference in vapour pressure. Thus if an insect were even slightly warmer than its surroundings, it would be able to evaporate water into completely saturated air.

(2) EXPERIMENTAL EVIDENCE.

Many experiments have been made which show the influence of climatic factors on insects, and from some of these the rate at which water is evaporated has been inferred. The experiments which have given the most important results may be divided into the following two classes:

(a) Those in which the actual amount of water evaporated is measured, or cal-culated from measurements of the insects' metabolism.

(b) Those in which the insects are exposed to various conditions of temperature and humidity, and by comparing the limiting conditions which permit survival, the rate of loss of water in the different experiments is inferred.

(a) Experimental measurements of the rate of loss of water.

Very few experiments have been made in which the water evaporated from insects has been collected and measured directly. It has usually proved more convenient to measure changes in the insects' weight, and, by indirect methods, to relate those changes to loss of water. Thus Gunn (1933) used measurements of the respiratory quotient of the cockroach, and Mellanby (1932b, c) analysed the bodies of several species of insects before and after his experiments. From these figures, they were able to calculate the amount of water which had been lost. Both workers found that metabolism alone caused practically no change in the weight in the insects, and, provided that the insects did not excrete, the loss in weight during starvation was all due to loss of water.

The experiments of Buxton (1930) on the mealworm, Gunn (1933) on the cockroach, and Mellanby (1932b, c, 1934a) on the mealworm, the bed-bug and the clothes-moth larva, all support the views expressed in the earlier sections of this paper. At one temperature and several humidities, the rate of loss of water in most cases is proportional to the saturation deficiency. In the case of the mealworm and the clothes-moth larva, it appears that a rise in temperature does not cause such great changes as it does in the other insects, and therefore when we compare the rate of evaporation at several temperatures we see that it is still much more closely proportional to saturation deficiency than to any other measure of humidity. At temperatures above 30° C. the body wall of the cockroach becomes more permeable to water (Ramsay, 1935), and the rate of evaporation increases enormously. The bed-bug also loses water more rapidly at high than at low temperatures (with the same saturation deficiency in each case), but this increase is of a much lower order from that obtained with the cockroach. It is presumably due to the greater rate of res-piration, accompanied by a more frequent opening of the spiracles—an explanation which must account for *part* of the increase of loss of water from the cockroach.

Experimental results also support the view that although the air in the tracheae is saturated when the spiracles are closed, when the spiracles are opened under dry conditions the air in the larger tracheae may become dry. Thus Buxton (1930) found that mealworms never lost more than 30 per cent. of their own weight in 28 days. They lost water at this maximum speed in dry air at 23° and 30° C.

(saturation deficiency 21 and 32 mm. respectively) and also in air which was 30 per cent. saturated at 30° C. (saturation deficiency 22 mm.); in moister air, they lost water at a rate approximately proportional to saturation deficiency (except in very moist air, where they absorb water into their bodies by some mechanism as yet not understood). It appears that this insect keeps its tracheal air moist when its spiracles are open except under extremely dry conditions. It is interesting to compare the results obtained with the bed-bug (Mellanby, 1932c). This insect loses water more rapidly in dry air than in moist, but, in proportion to the saturation deficiency, the rate of loss is greatest in air which is 90 per cent. saturated. When bugs are starved for a month under different conditions of humidity, the rate of respiration (and the extent to which the spiracles are opened) decreases equally at each humidity as time goes on. In moist air, the rate of loss of water falls off proportionately, as though the limiting factor were the extent to which the spiracles were opened. In dry air, however, the rate of loss remains constant—the limiting factor is not the extent to which the spiracles are opened, but the rate at which water vapour enters the tracheae from the body. The results obtained by Bodine (1921) with hibernating nymphs of the grasshopper *Chortophaga viridifasciata* support the same argument. When the saturation deficiency of the air is greater than 20 mm., the air in the tracheae apparently becomes fairly dry, as beyond that point the rate of loss of water, in proportion to the saturation deficiency, decreases considerably.

(b) Conditions limiting insect survival.

The experiments classified under this heading are very numerous, and very varied. They include those in which insects were isolated and subjected to several combinations of temperature and humidity, and either the time of survival or else the maximum temperature permitting survival was found. They also include all those breeding experiments where it was found that certain combinations of temperature and humidity proved favourable, and others proved unfavourable.

One important point has been frequently forgotten by workers in interpreting their results. If a result is attributed to the effects of humidity, and conclusions are drawn from it, then we must be sure that all other variables except humidity have been excluded. For instance, if we wish to discover how humidity governs loss of water by finding the length of exposure required to kill insects at various humidities, then we must be sure that death *in all cases* is due to desiccation and to desiccation only. Lack of attention to this fact is responsible for the present confusion. Phenomena due partly to starvation, etc., have too often been attributed to desiccation and greatly confused the issue. This point will be made clearer by reference to examples below.

Among the earliest workers to realise that the rate of loss of water from insects was probably governed by saturation deficiency were Bacot and Martin (1924), who worked on the effects of temperature and humidity on the survival of the flea. They removed batches of fleas from a culture, and kept them at a number of combinations of temperature and humidity. They found that at one temperature, 32° C., the length of survival was inversely proportional to saturation deficiency except in

moist air, where, presumably, death was not due to desiccation. They also found that fleas died more quickly at a saturation deficiency of 10 mm. at 32° C. than they did at a temperature of 21° C. and the same saturation deficiency. This is explained principally by the fact that the rise in temperature would increase the rate of metabolism, cause the spiracles to be opened more frequently, and so allow more water to be lost. The more recent work of Leeson (1932) on the same species of flea gives results that appear at first sight to conflict with those of Bacot and Martin. But whereas Bacot and Martin used fleas removed from a culture in which a rodent was present, so that they had had a chance of feeding, Leeson's fleas were used immediately after emerging from their cocoons. Presumably Bacot and Martin's fleas died of desiccation, and Leeson's of starvation combined in some cases with desiccation.

Other experiments of the type done by Bacot and Martin, such as those of Jones (1930) with the bed-bug, give the same result, provided that only those in which desiccation was the *sole* cause of death are considered.

Some experiments have been made to find what temperatures insects are able to survive for definite periods (say 1 hour or 24 hours), under different conditions of atmospheric humidity. Some results, taken from the work of Mellanby (1932a) are given on Fig. 2. The curves represent the highest temperatures at which certain insects survived for 24 hours, and lines of equal saturation deficiency are drawn on the same figure. It will be seen that certain of the insects, for instance the adult flea and the mealworm, die at the same temperature in dry and in moist air. The explanation of this is presumably that, although these insects are losing much more water in dry air than in moist (which conclusion is supported by the work of Bacot and Martin described above), yet they always lose water fairly slowly. At the end of 24 hours, even at temperatures just below the thermal death point and in completely dry air, they still contain sufficient water to live. These insects are killed by the heat itself. The other insects all die in moist air at approximately the same temperature, where again heat alone is the cause of death; they die at much lower temperatures in dry air. This means that in dry air, one factor causing death must be desiccation. Now, if desiccation causes death, does death always occur when a definite amount of water has been lost? In other words, can these insects survive just so long as the amount of water in their bodies exceeds some definite minimum? If this is the case, and if the rate at which water is lost is proportional to the saturation deficiency, then the curves on Fig. 2 giving the highest survival temperatures for the louse, flea larva, etc., should coincide with curves of equal saturation deficiency. It will be seen at once that they do not, and this might be taken to mean that the rate of loss of water is not proportional to saturation deficiency. Actually there is a much simpler explanation. All the insects except the flea larvae are exposed to two unfavourable factors, heat and desiccation, and they die at a lower temperature in dry than in moist air, *partly* due to desiccation, and *partly* also due to the effects of heat. Then these insects all have mechanisms for closing their spiracles. As the temperature rises, their spiracles will be more frequently opened, and so they will lose the same amount of water at a lower saturation deficiency. The flea larva was the only insect

exposed to desiccation alone, for it died in dry air at such low temperatures. Incidentally, the flea larva has no spiracle closing mechanism, and it will be seen that (except in moist air where death must be due to the heat) the curve of highest
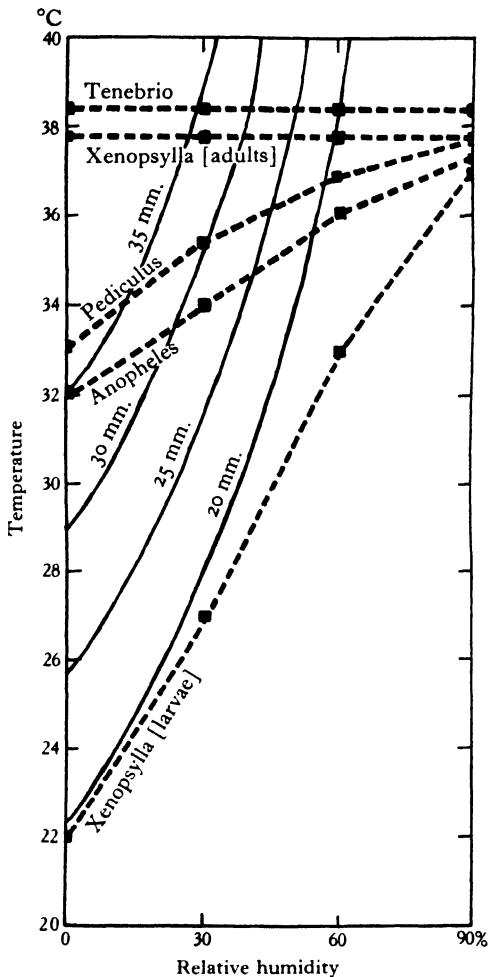
Fig. 2. The highest temperatures which certain insects were able to survive for exposures of 24 hours, with air of different humidities. Lines of equal saturation deficiency have been drawn on to the same figure.

survival temperature is parallel to the curve for 20 mm. saturation deficiency. Thus the evidence is that, where desiccation *alone* is the cause of death, water is lost at a rate proportional to saturation deficiency. Where death is not due to desiccation alone, it is impossible to draw conclusions as to the factors governing the rate of loss of water.

There are a great number of experiments in which the limiting conditions of temperature and humidity under which insects' eggs will hatch have been found, and Buxton (1931, 1932) has shown that in certain cases these figures support the theory that the rate at which water is lost is proportional to saturation deficiency. But other figures have been published which appear at first to be contrary to that theory. Among these are those of Maercks (1933 a). He exposed batches of eggs of *Habrobracon juglandis* to a great variety of different temperatures and humidities, and among other things discovered the proportion of eggs hatching under each condition. At first sight, these figures appear to bear no relation to saturation deficiency, and Maercks infers that relative humidity governs the rate of water loss. But what he has not realised is that, if we are considering the *rate* of loss of water, we must take the length of the experiment—*i.e.* the time the eggs take to hatch—into account. His eggs hatched in 24 hours at 30° C. and they took as long as 286 hours at 13·4° C. Now if the rate of loss of water from these eggs is proportional to the saturation deficiency of the air, then the actual amount of water lost should be proportional, not to the saturation deficiency, but to the product of the time of exposure and the saturation deficiency. On Fig. 3 some of Maercks' results are given. The black circles show under what conditions the experiments were performed. The figures inside the rectangles give the product of the saturation deficiency and the time the eggs took to hatch. The other figures show what proportion of eggs failed to hatch. Thus at 16·25° C. and 5·5 per cent. relative humidity, the saturation deficiency was 13·0 mm., and the eggs hatched on an average in 141 hours. The product of these figures is 184[1]. Under these conditions 89 per cent. of the eggs failed to hatch, and 11 per cent. hatched. Where crosses are shown on the figure, no eggs hatched. Now it will be seen that below 13° and above 38° C., the eggs were all killed by cold and heat respectively. At intermediate temperatures, the proportion hatching bears some relation to the atmospheric humidity. It also appears that, roughly speaking, the higher the product of the saturation deficiency and of the time taken by the eggs to hatch, the lower the proportion of eggs which hatched. A perfect relationship is not obtained, but it is as close as could be expected for figures of this kind. The dotted lines drawn on Fig. 3 enclose the points at which a high proportion of the eggs hatched, and the figures inside the rectangles within these dotted lines are for the most part lower than those outside. The relationship is least apparent at high temperatures, where doubtless the eggs were subjected to the two unfavourable conditions of heat and desiccation. But where the only unfavourable factor was desiccation, the hatching of these eggs appears to be controlled by the product of the saturation deficiency of the air and the time taken by the eggs to hatch. The rate of loss of water, therefore, must be governed by the saturation deficiency.

A similar conclusion appears from the results of Mellanby (1933) on the conditions limiting metamorphosis of flea larvae. At 18°, 22° and 29° C., these larvae only pupate when the air has a relative humidity of 60 per cent. or higher, and at 35° C. they require a humidity of 80 per cent. These points have quite different saturation deficiencies, but when the time taken by the larva to pupate at any

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[1] For convenience, the products have all been divided by 10.

temperature is multiplied by the limiting saturation deficiency for the same temperature, the product is practically the same in each case. In other words, the saturation deficiency is again seen to govern the rate of loss of water.

Reference once more to Fig. 3 reveals another important fact. In essentials, this figure is similar to the wheel diagram, first drawn by Pierce (1916). (Actually,
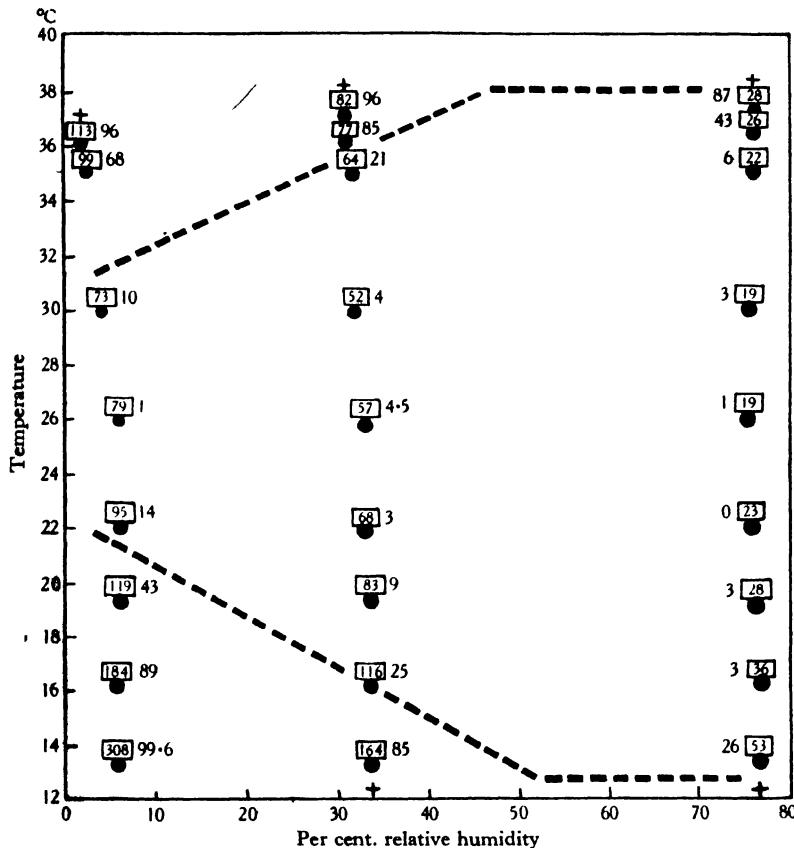
·Fig. 3. The effects of various temperatures and humidities on the hatching of eggs of *Habrobracon juglandis* (figures derived from Maercks, 1933 a). The black dots represent the points at which the experiments were made. The figures inside the rectangles give the products of the saturation deficiency (in mm.) and the time which the eggs took to hatch (in hours). The other figures show what proportion of the eggs failed to hatch.

Pierce, Maercks and many others have found that very moist air was also unfavourable. This cannot be due to *loss* of water, and so will not be considered here.) Pierce found that certain combinations of temperature and humidity were unfavourable for the development of the cotton-boll weevil and, like *Habrobracon*, at certain middle "optimum" temperatures, a low humidity is less unfavourable than is the same (relative) humidity at either a higher or a lower temperature. The

explanation of this fact can be found by referring to Fig. 3. The extent to which the
eggs are dried, that is, the product of the "dryness," or saturation deficiency, and
the period of exposure of the eggs, is represented by the figures inside the rect-
angles. It will be seen that, at one relative humidity, the products decrease as the
temperature rises from 14° to 30° C., but that above that temperature the products
increase once more. In other words, at middle temperatures the eggs are exposed to
less drying than at high or at low temperatures. The explanation of this is that, as
the temperature rises, the saturation deficiency increases, but the time taken for
development decreases. Up to 30° C., the time taken for development decreases
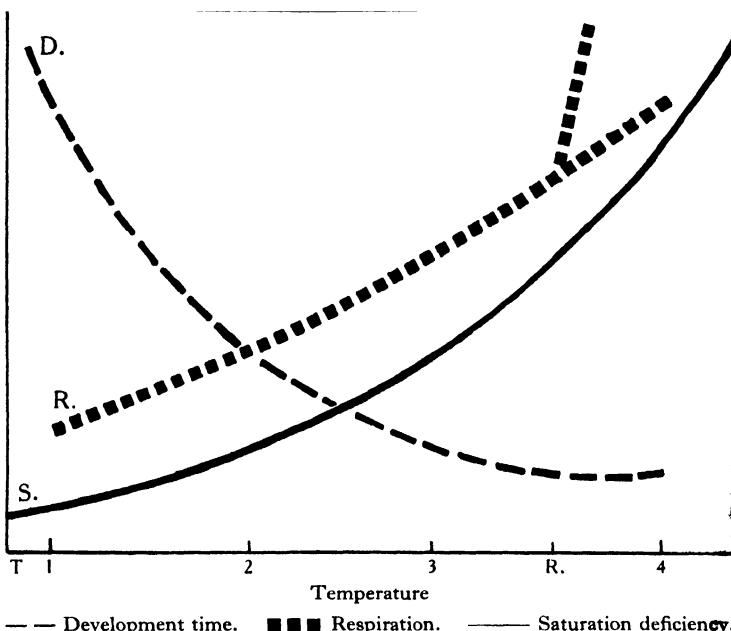
Fig. 4. The effects upon water loss from insects of: (1) the time taken by insects to develop (D);
(2) the rate of respiration (R); and (3) saturation deficiency (S). For explanation see text.

more rapidly than the saturation deficiency increases, but above 30° C. the satura-
tion deficiency increases more quickly. Those two facts alone explain the results
obtained with the *Habrobracon* eggs and the flea larvae—both cases where the issue
is not complicated by the presence of mechanisms for closing the spiracles.

Fig. 4 is a synthetic diagram showing how the various factors influence the loss
of water from insects. The three curves show the effects of temperature upon
humidity (saturation deficiency = S), the rate of development (length of expo-
sure = D) and respiration (spiracular opening = R). The curve R also shows the
effect of high temperature upon the permeability of the body surface, as found by
Ramsay (1935) for the cockroach. The saturation deficiency curve may be derived
from the figures given in collections of physical data. The curve showing how tem-

perature affects the time taken for development is similar to many which have been described by a number of workers (for instance, see Janisch and Maercks, 1933). The respiration curve R is similar to that published by Gunn (1933) except for the way in which it divides at TR. At this point, the steeply rising curve is intended to show the effects of both respiratory movements and the increased permeability of the body wall which may occur at higher temperatures.

T_1 shows where development is stopped by cold, and T_4 where it is stopped by heat. Between these two temperatures the effects of humidity may be seen. As the temperature rises from T_1 to T_2, the time taken for development decreases more rapidly than the saturation deficiency increases, so their product decreases—and the unfavourable effect of low humidity decreases also. Between T_2 and T_3 is the "optimum" zone of temperature, where the product of the time of development and the saturation deficiency is lowest. Here a low humidity has its least harmful effect. Above T_3, the length of development decreases very slowly (it may even increase near T_4) and the saturation deficiency increases rapidly, so once more their product rises. Fig. 4 provides a simple explanation of the occurrence of optimum temperatures at which insects can develop over the widest range of humidities. The actual position of the optimum depends on the relations between S, D and R, which are different for each species.

In cases like that of the flea larva and the *Habrobracon* eggs, only the curves S and D need be considered. In many insects, however, the rate of respiration and its effects on spiracular opening (curve R) complicate the picture. With them, an increase in the rate of respiration alone, with no change in saturation deficiency, will cause an increase in the rate of loss of water. A change in the permeability of the body wall would have the same effect. In studying the effects of climatic conditions on such insects, all three factors shown in Fig. 4 must be taken into account. In experimental work, it is often possible to keep two of the variables constant, and find the effects of the third by itself. In the field, when we wish to compare the effects of the climatic conditions of two areas on one species of insect, as well as considering the effects of saturation deficiency, we must take into account how temperature—and also humidity—influences the rates of development and metabolism. It sometimes appears that a useful comparison between the climates of two areas (as they affect insects) may be obtained from the saturation deficiencies (Brooks, 1917), but it must be remembered that the other factors also have important effects on insects.

(3) The "saturation deficiency law."

In the light of the results described in the previous pages, we can express the ways in which atmospheric humidity affects the rate of loss of water from insects in the form of a law. The law may be stated thus: "If individual insects of the same species, in identical morphological and physiological states, are exposed to atmospheres with different humidities, then the rates at which the insects lose water will be proportional to the saturation deficiency, provided that the saturation deficiency is not above a maximum figure which is peculiar to the species. From this it follows

that when insects are killed by desiccation, the length of life of the insects will be inversely proportional to the saturation deficiency of the air."

There are two corollaries to the law:

(1) "If the rate of loss of water from different individual insects does not appear to be proportional to the saturation deficiency of the air, then their morphological and physiological states cannot be the same."

(2) "When insects are exposed to atmospheres of different humidity, if there appears to be no direct relation between the length of time which they survive and the saturation deficiency, then other lethal factors, besides desiccation, must be at work."

Perhaps the most important implication of the "law" as stated above is contained in the first corollary. This means that we are now able to explain results which many workers described as "conflicting with the saturation deficiency hypothesis." The results no longer conflict, and our better understanding of the factors governing evaporation make it possible to use these results to obtain both morphological and physiological information.

V. SUMMARY.

This article deals with the way in which climatic conditions and insect metabolism affect the rate at which water is evaporated from insects' bodies.

It appears that practically all the water evaporated from insects is lost from the tracheal system. At high temperatures the body wall of the cockroach becomes permeable to water.

Experimental results may be explained by assuming that when the spiracles are closed, the air in the tracheae is saturated, and in most cases it remains saturated when the spiracles are opened. In some cases, however, particularly in very dry air, the air in the tracheae becomes somewhat dry. The evidence for these suppositions is mainly indirect, but appears reasonably conclusive.

The proportion of dry matter in insects, and the ratio of bound water to free, cannot, of themselves, affect the resistance of insects to desiccation. The best protection against desiccation appears to be an efficient mechanism for closing the spiracles, together with the ability to conserve water in the rectum and pass dry excreta.

Experiments in which the rate of loss of water is measured in air of various humidities show that the rate is more nearly proportional to the saturation deficiency of the air than to any other measure of humidity. As the temperature rises, the rate of loss at one saturation deficiency sometimes increases, because the insect opens its spiracles more frequently.

Experiments to determine the conditions of temperature and humidity which limit survival and breeding of various species support the view that the rate of loss of water is proportional to saturation deficiency. In work of this kind, it is important that results be attributed to the effects of humidity only when humidity alone is the causal factor.

The occurrence of optimum temperatures, at which insects can develop over a wider range of humidity than at higher or lower temperatures, is discussed, and a simple explanation is suggested.

The ways in which the various environmental and internal factors influence the rate of loss of water are discussed, and the law governing the rate of evaporation of water from insects is stated in a revised form, and its implications are defined.

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NEUERE ERGEBNISSE UND PROBLEME AUS DEM GEBIET DER OSMOREGULATION WASSERLEBENDER TIERE

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GLIEDERUNG.

I. EINLEITUNG.

EINE ausführliche Schilderung der Osmoregulation wasserlebender Tiere habe ich vor vier Jahren in dieser Zeitschrift gegeben (Schlieper, 1930). Wenn ich bereits heute wieder an der gleichen Stelle über dasselbe Problem berichten kann, so liegt dies in der schnellen Fortentwicklung begründet, die dieses interessante Teilgebiet der biologischen Forschung in dem genannten Zeitraum erlebt hat. Vieles was ich seinerzeit als Hypothesen andeutete, ist längst durch die neuen Ergebnisse bestätigt bezw. überholt worden, andere Probleme treten nunmehr in den Vordergrund des Interesses. Es erscheint deshalb wünschenswert dem damaligen ersten Artikel jetzt einen zweiten als Fortsetzung folgen zu lassen.

Ich werde im Folgenden die Regulation des osmotischen Druckes (der Molarkonzentration) der Körperflüssigkeiten bei den wasserlebenden Evertebraten und den Fischen besprechen. Den Wasserhaushalt der Amphibien brauche ich nicht in den Rahmen meiner Arbeit einzubeziehen, da erst kürzlich Adolph (1933) den Frosch in dieser Richtung monographisch behandelt hat. Ebenso werde ich über das osmotische Verhalten einzelner Zellen nicht berichten, um den Umfang meiner Arbeit nicht zu gross werden zu lassen[1]. Aber auch trotz dieser Beschränkung ist

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[1] Ich verweise auf die Monographie von Lucké und McCutcheon (1932) sowie auf die Arbeiten von Jacobs (1933) und seiner Schule.

der von mir gewählte Rahmen meines Referates ausserordentlich gross, denn das Problem der Osmoregulation hängt seinerseits wieder mit verschiedenen anderen biologischen Grundproblemen eng zusammen. Da die Osmoregulation eines Wassertieres natürlicherweise von der Art der Durchlässigkeit der Körperober- flächen stark beeinflusst wird, ergeben sich Beziehungen zum allgemeinen Per- meabilitätsproblem. Da anorganische Salze einen wesentlichen Teil des osmotischen Druckes der Körperflüssigkeiten verursachen, sind die osmoregulatorischen Vor- gänge bei allen Organismen eng mit dem Mineralhaushalt verbunden. Weil weiterhin die Exkretionsorgane bei einer Reihe von Wassertieren wichtige osmo- regulatorische Aufgaben erfüllen, haben auch Untersuchungen über die osmo- regulatorischen Leistungen dieser Organe bei Wassertieren wesentliche Beiträge zum Verständnis des Nierenproblems überhaupt geliefert. Ausserdem lässt sich nachweisen, dass die osmoregulatorischen Fähigkeiten der Wassertiere für die Verbreitung der einzelnen Arten innerhalb der Gewässer unserer Erde (Meer, Brackwasser, Süsswasser) bestimmend gewesen sind, demnach haben auch die Tiergeographie und die Tierökologie ein grosses Interesse an den Ergebnissen unseres Forschungsgebietes. Schliesslich ist das Osmoregulationsproblem selbst- verständlich ein Teilproblem der allgemeinen Physiologie. Wir sind deshalb bestrebt, die osmoregulatorischen Vorgänge in den Gesamtstoffwechsel und den Energiehaushalt der Tiere einzuordnen. Es haben sich im Verlauf derartig gerichteter Untersuchungen schon interessante Beziehungen zwischen der Osmoregulation, dem Wassergehalt (Hydratationsgrad) der Gewebe und der Atmung ergeben, Zusam- menhänge, die ihrerseits zum Teil wieder gewisse biologische und morphologische Regeln erklären (z. B. das Aufsuchen sauerstoffreicher Gebiete beim Vordringen mariner Tiere in das Süsswasser, die Reduktion von Atmungsorganen mit zuneh- mendem Salzgehalt im Aussenmedium).

II. DIE OSMOREGULATORISCHEN FÄHIGKEITEN DER WASSERTIERE.

Die Aufrechterhaltung des normalen kolloidalen Zustandes des Protoplasmas erfordert es, dass die Molarkonzentration der Körperflüssigkeiten bei allen Tieren mehr oder weniger konstant ist. Deshalb sind überall da osmoregulatorische Mechanismen ausgebildet, wo durch äussere Einflüsse—Verdunstung bei den ·Landtieren, andersartige Zusammensetzung des Aussenmediums bei den Wasser- tieren—diese notwendige Konstanz des Innenmediums bedroht wird. Natürlich steht die Leistungsfähigkeit der osmoregulatorischen Einrichtungen bei den ein- zelnen Tiergruppen in Beziehung zu den biologischen Erfordernissen. Das lässt sich bei Land- und Wassertieren nachweisen. Ein Landtier, wie der Regenwurm (*Lumbricus*), dessen Gänge in der Erde gelegentlich bei starkem Regen mit Wasser gefüllt werden, kann ohne Schädigung wochenlang in Süsswasser gehalten werden (Focke, 1930). Dagegen geht die Weinbergschnecke (*Helix pomatia*), die in freier Natur kaum einmal in die Gefahr des Ertrinkens gerät, sehr bald durch osmotische Wasseraufnahme (nicht durch Ersticken) zugrunde, wenn man sie im Laboratorium in einem mit Süsswasser gefüllten Behälter einschliesst (Jordan-Hirsch, 1927;

Courtois und Duval, 1927). Einen Überblick über die osmoregulatorischen Lei-
stungen der Wassertiere gibt die Tabelle I. Während die *Süsswasserwirbellosen*

Tabelle I. *Gefrierpunktserniedrigung bezw. Molarkonzentration des Innenmediums* (J)
der wasserlebenden Tiere im Vergleich zu ihrem natürlichen Aussenmedium (A).

Marine Evertebraten	$J = A$
Marine Elasmobranchier	$J > A$
Marine Teleostier	$J < A$
Süsswassertiere	$J > A$

ein gegenüber ihrem Aussenmedium hypertonisches Innenmedium aufrecht-
erhalten, haben die *marinen Wirbellosen* im Verhältnis zum Meerwasser nahezu
isotonische oder nur wenig hypertonische Körpersäfte. Danach könnte es scheinen,
als ob unter den Wirbellosen nur die im Süsswasser heimischen und die marinen
Einwanderer in das Süsswasser homoiosmotische Eigenschaften besässen. Das ist
aber nicht der Fall, denn zahlreiche euryhaline, marine Evertebraten, die im Meer
und in dem Brackwasser der Flussmündungen und Nebenmeere (Ostsee) leben,
haben osmoregulatorische Fähigkeiten (Schlieper, 1929, 1930; Pantin, 1931;
Bogucki, 1932; Schwabe, 1933)[1]. Das Gleiche liess sich auch für gewisse marine
euryhaline Wirbellose tropischer Meeresküsten nachweisen, deren Wasser nach
heftigen Regenfällen eine starke Abnahme des Salzgehaltes aufweist (Dakin und
Edmonds, 1931). Alle diese euryhalinen Evertebraten besitzen in verdünntem
Seewasser ein hypertonisches Innenmedium. Sie sind eigenartigerweise sogar
schon bei Salzgehalten ihres Aussenmediums homoiosmotisch, welche die Konzen-
tration der Körperflüssigkeiten der ihnen verwandten Süsswasserarten oft weit
übersteigen. Die Brackwasserkrabbe *Carcinus maenas* hat z. B. schon in Brackwasser
von 20 °/$_{oo}$ Salzgehalt ein hypertonisches Innenmedium. Der Unterschied in den
Molarkonzentrationen des Innen- und Aussenmediums kommt dabei durch einen
höheren Gehalt an anorganischen Salzen (im Wesentlichen Chloriden) im Innen-
medium zustande. Führt man also einen stenohalinen und einen euryhalinen
marinen Wirbellosen aus Meerwasser in Brackwasser über, so sinkt zunächst bei
beiden Tieren die Konzentration der in den Körpersäften vorhandenen gelösten
Salze. Während aber bei der stenohalinen poikilosmotischen Art ein Ausgleich
zwischen Innen- und Aussenmedium zustande kommt, bleibt das Innenmedium
der euryhalinen fakultativ homoiosmotischen Art hypertonisch gegenüber dem
neuen Aussenmedium (siehe Abb. 1).

Die *Süsswasserevertebraten* sind—wie gesagt—im Besitze osmoregulatorischer
Einrichtungen. Da das Süsswasser infolge seines geringen Elektrolytgehaltes als
inneres Medium nicht geeignet ist, halten diese Tiere in ihren Körpersäften einen
osmotischen Druck aufrecht, welcher den des Aussenmediums um das Vielfache
übersteigt. Erhöht man bei irgendwelchen Süsswasserevertebraten die Salzkonzen-

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[1] Die Untersuchungen von Hykes (1930) an Ctenophoren beweisen nicht die Existenz homoi-
osmotischer Eigenschaften bei diesen Tieren. Die gleichen Gewichtsänderungen nach Überführung
in hypotonische Lösungen, wie sie dieser Autor bei *Beroe* feststellte, lassen sich auch bei nachweislich
poikilosmotischen Evertebraten beobachten.

tration im Aussenmedium, so versagen ihre osmoregulatorischen Mechanismen bald. Bei Individuen, die einige Zeit in isotonischem Salzwasser gelebt haben, lässt sich jedoch meistens eine deutliche Hypertonie der Körpersäfte gegenüber dem Aussenmedium nachweisen. Das Blut des Flusskrebses (*Potamobius fluviatilis*) hat z. B. normalerweise eine Gefrierpunktserniedrigung von 0,80 Grad; führt man ihn aber in blutisotonisches Brackwasser über, so steigt die Gefrierpunktserniedrigung seines Blutes allmählich auf 1,00 Grad (Herrmann, 1931). Die osmoregulatorischen Mechanismen des Flusskrebses sind also in isotonischem Salzwasser immerhin noch etwas in Tätigkeit. Erhöht man jedoch den Salzgehalt im Aussenmedium noch weiter, so verlieren die osmoregulatorischen Mechanismen vollkommen ihre Funktionsfähigkeit: Innen- und Aussenmedium werden isotonisch.

Die *Gewebe* sind bei den Süsswasserwirbellosen—und ebenso wahrscheinlich bei den Fischen des Süsswassers—in gewisser Weise bei der Osmoregulation beteiligt. Bei hungernden Individuen kompensieren sie durch Abgabe von Salzen
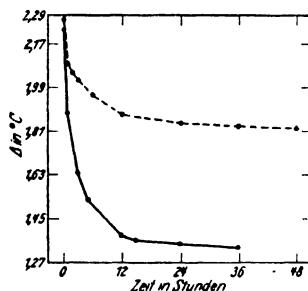
Abb. 1. Änderung der Blutkonzentration bei *Maja verrucosa* und *Carcinus maenas* nach Überführung in verdünntes Seewasser (25 °/₀₀ Salzgehalt, Gefrierpunktserniedrigung $\Delta = 1,33°$ C.). —— *Maja*; – – – *Carcinus* (nach Schwabe).

den durch Exkretion etc. bedingten Salzverlust der zirkulierenden Körpersäfte. Ausserdem können sie eine Verdünnung des Blutes durch Bindung überschüssigen Wassers als Quellungswasser verhindern. Infolge dieser "Innenregulation" ist die Molarkonzentration des Blutes der Süsswassertiere auch dann konstant, wenn grössere Salzverluste durch Blutungen infolge von Verwundungen vorkommen oder wenn grosse Wassermengen durch eine Wunde von aussen osmotisch eindringen. Bei längerem Hungern nimmt jedoch die Molarkonzentration des Blutes bei allen Süsswassertieren allmählich ab (Scholles, 1933; Huf, 1933).

Die osmoregulatorischen Fähigkeiten der Fische sind oft Gegenstand eingehender Untersuchungen gewesen. Während die Süsswasserfische ein gegenüber dem Aussenmedium hypertonisches Innenmedium aufrechterhalten, arbeiten die osmoregulatorischen Einrichtungen der marinen Teleostier grade in umgekehrter Richtung, denn das Blut dieser Fische ist hypotonisch im Verhältnis zum Meerwasser. Im Gegensatz hierzu ist wieder das Blut der marinen Elasmobranchier auf Grund eines Harnstoffgehaltes von 2–3 % schwach hypertonisch gegenüber dem Meerwasser. Neuere Untersuchungen haben uns gelehrt, dass auch das Blut der

tropischen Süsswasserelasmobranchier einen im Vergleich zu anderen Tieren hohen Harnstoffgehalt hat (H. W. Smith, 1931) (siehe Tabelle II).—Die Arbeiten der

Tabelle II. *Harnstoff- und Chloridgehalt des Blutes verschiedener Wassertiere.*

	Aussen-medium $\Delta°$ C.	Serum $\Delta°$ C.	Serum-Harnstoff-N mg %	Serum-Cl mg/100 ccm
Süsswasserteleostier	0,0	0,70	10–30	400
Marine Teleostier	1,85	0,80	10–30	500
Süsswasserelasmobranchier	0,0	1,0	300	600
Marine Elasmobranchier	1,85	1,95	1000	800

letzten Jahre bestätigen auch bei den Fischen die schon bei der Besprechung der wasserlebenden Evertebraten erwähnte Regel, dass mit zunehmender Euryhalinie die osmoregulatorischen Fähigkeiten der Wassertiere grösser werden. Dabei können ebenso wie bei den Wirbellosen ganz nahe verwandte Arten grosse Unterschiede aufweisen. So findet sich z. B. innerhalb der Teleostiergattung *Blennius* eine marine Art, *Blennius pavo*, die ohne Schädigung unmittelbar in Süsswasser überführt werden kann, während zwei andere Arten, *B. tentacularis* und *B. gattorugine*, nur in Meerwasser lebensfähig sind. Zwischen diesen beiden Extremen gibt es dann noch eine Art von mittlerer Euryhalinie, *B. pholis*, welche in Meer- und Brackwasser nicht aber in reinem Süsswasser zu finden ist (Sonnery und Tchang-Si, 1931).—Dass verschiedene Grade der Euryhalinie tatsächlich in Unterschieden der osmoregulatorischen Leistungsfähigkeit begründet sind, zeigten u. a. Untersuchungen an *Conger vulgaris*. Dieser marine Teleostier ist in Süsswasser nicht lebensfähig, er erträgt aber immerhin eine Verdünnung des Meerwassers bis zu einem Zehntel. Die Blutkonzentration nimmt wenig ab, solange das Aussenmedium nicht über das Zehnfache verdünnt wird. Setzt man jedoch den Salzgehalt im Aussenmedium noch weiter herab, so sinkt auch der Salzgehalt des Blutes stärker, gleichzeitig schwellen die Versuchstiere an und sterben (Margaria, 1931). Die Ursache des Absterbens von *Conger* in stark verdünntem Seewasser ist also das Versagen seiner osmoregulatorischen Einrichtungen in diesem Medium. Untersucht man die osmotische Resistenz von jüngeren und älteren Individuen einer euryhalinen Art, so findet man häufig, dass die jüngeren Individuen gegenüber Salzgehaltsschwankungen im Aussenmedium empfindlicher sind als die älteren Exemplare. Neuere Versuche weisen wiederum daraufhin, dass auch diese Unterschiede in einer verschiedenen Leistungsfähigkeit der osmoregulatorischen Mechanismen begründet sind. So zeigen nämlich junge Aale, welche eben aus dem Meer in die Flüsse eingewandert sind, beim Überführen aus Süsswasser in Seewasser bedeutend grössere Veränderungen des osmotischen Druckes im Innenmedium, als bei den erwachsenen Aalen im gleichen Fall zu beobachten ist. Während die Konzentration des Blutes bei einem ausgewachsenen Aal im Seewasser maximal um 24 % höher ist als im Süsswasser, beträgt die entsprechende Differenz für den osmotischen Druck der Körpersäfte bei den genannten jungen Aalen 41 % (Firly, 1932).

Als Ergänzung möchte ich noch einige Beziehungen zwischen der *Mineralregulation* und der Osmoregulation bei den wasserlebenden Tieren erörtern. Die anorganischen Salze verursachen bei allen Wassertieren mit Ausnahme der marinen Elasmobranchier den Hauptteil der Molarkonzentration der Körperflüssigkeiten. Dementsprechend wird in den meisten Fällen die Osmoregulation durch eine Regulation des Mineralgehaltes der Körpersäfte bewerkstelligt. Der Mineralgehalt der Körpersäfte der Süsswassertiere ist fast vollkommen unabhängig von der Zusammensetzung des Aussenmediums. Ähnlich verhalten sich die marinen Fische, aber auch bei den marinen Evertebraten stimmt die mineralische Zusammensetzung der Körpersäfte im allgemeinen nicht genau mit der des Seewassers überein, selbst dann nicht, wenn Innen- und Aussenmedium isotonisch sind (siehe Tabelle III). Während die Körperflüssigkeiten der Mollusken und Echinodermen

Tabelle III. *Mineralgehalt der Körperflüssigkeiten verschiedener Wassertiere (nach Bethe und Berger, Bialaszewicz, McCallum, Scholles).*

Art	Cl	Atomgehalt/Cl (Cl = 100)			
	mg/ccm	Na	K	Ca	Mg
Meerwasser (Neapel)	22,27	88,0	1,97	2,04	9,85
Aplysia punctata	22,17	91,5	1,87	2,10	8,8
Sipunculus nudus	23,6	—	1,88	1,76	6,2
Carcinus maenas	21,6	—	2,35	2,31	4,35
Palinurus vulgaris	21,6	100,8	4,55	4,55	2,5
Potamobius fluviatilis	6,91	—	2,93	6,05	0,93
Scyllium canicula	11,20	—	3,75	1,44	2,13
Gadus callaris	6,22	—	5,76	2,32	1,38

in Bezug auf ihre mineralische Zusammensetzung noch ziemlich seewasserähnlich sind, zeigen die Blutsera der höheren marinen Evertebraten weitgehende Abweichungen, insbesondere ist bei manchen Anneliden und Crustaceen der Magnesiumgehalt der Körperflüssigkeiten bedeutend niedriger als der des Meerwassers (Bethe und Berger, 1931; Bialaszewicz, 1933).

III. OSMOREGULATORISCHE LEISTUNGEN DER EXCRETIONSORGANE.

Die primäre Aufgabe der Exkretionsorgane bei zahlreichen Süsswassertieren ist die *Wasserausscheidung*. Viele Süsswassertiere nehmen dauernd osmotisch Wasser durch die Haut auf, während ihre Exkretionsorgane kontinuierlich das überschüssige Wasser ausscheiden und dadurch Wasser und Salze im Organismus in einem dynamischen Gleichgewicht erhalten. Der Wasserstrom, der auf diese Weise den Körper dieser Tiere durchfliesst, spült gleichzeitig die stickstoffhaltigen Endprodukte des Stoffwechsels hinaus. Die Voraussetzung für eine derartige Arbeitsweise der Exkretionsorgane—der osmotische Wassereinstrom durch die Haut—fehlt aber natürlich bei den marinen Evertebraten und Teleostiern. Deshalb ist bei diesen zuletzt genannten Tiergruppen eine osmoregulatorische Funktion der Exkretionsorgane entweder überhaupt nicht vorhanden, oder sie ist doch stark abgeändert.

Die Richtigkeit dieser Anschauung wird durch zahlreiche morphologische und physiologische Beobachtungen bewiesen.

Das Auftreten und die Tätigkeit der pulsierenden Vakuolen bei den Protozoen zeigen eine Steigerung im Süsswasser gegenüber dem Meerwasser (siehe z. B. Kitching, 1934). Die alten Befunde von Marchal und Rogenhofer, nach denen bei vielen Crustaceen die Süsswasserformen längere Nephridialkanäle aufweisen als ihre Verwandten im Meere, sind neuerdings durch die Untersuchungen von Schwabe (1933) an *Gammarus* bestätigt und erweitert worden. Der Nephridialkanal der Antennendrüsen des süsswasserlebenden *Gammarus pulex* ist nach diesem Autor fast doppelt so lang als bei dem marinen *G. locusta* (siehe Abb. 2). Ähnlich verhalten sich viele Turbellarien sowie der euryhaline Polychaet *Nereis diversicolor* im Vergleich zu der mehr stenohalinen *N. pelagica*. Ausnahmen von dieser Regel
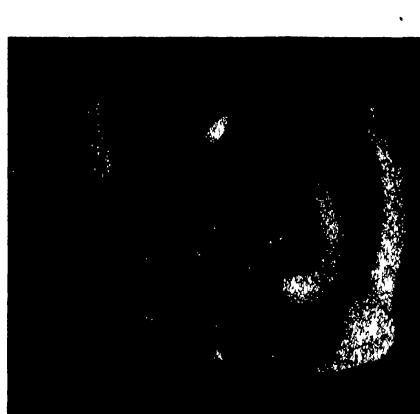
(a)   (b)

Abb. 2. Modelle der Exkretionsorgane (Antennendrüsen) von *G. locusta* (a) und *G. pulex* (b). Vergr. etwa 185-fach. *CS* = Coelomsäckchen, *NK* = Nephridialkanal (nach Schwabe).

bilden die marinen Rotatorien und einige Crustaceen, wie z. B. *Telphusa fluviatile* (Marchal, 1892) und *Mysis relicta* (Vogt, 1933).

Vergleichen wir im Einzelnen—um an einem Beispiel die Beziehungen näher zu erläutern—den Bau der Exkretionsorgane des marinen Hummers (*Homarus vulgaris*) und des süsswasserlebenden Flusskrebses (*Potamobius fluviatilis*), so können wir mehrere Unterschiede feststellen, die auf die Wirkung des verschiedenen Aussenmediums zurückzuführen sind. Die Antennendrüsen (Exkretionsorgane) des Flusskrebses lassen vier Abschnitte erkennen: (1) einen Filtrationsabschnitt— das Coelomsäckchen, (2) einen Sekretionsabschnitt—das Labyrinth, (3) einen Rückresorptionsabschnitt—das Harnkanälchen und (4) die Harnblase mit einem kurzen Ausführungsgang (siehe Abb. 3). Beim Hummer fehlt der Rückresorptions-teil ganz, und ausserdem ist bei ihm im Gegensatz zum Flusskrebs der Sekretions-abschnitt ausserordentlich stark entwickelt. Die Arbeitsweise der vier Abschnitte

stelle ich mir—wie ich schon durch die obigen Bezeichnungen ausgedrückt habe—
folgendermassen vor. Bei dem Flusskrebs wird entsprechend dem osmotischen
Wassereinstrom durch die Haut und dem dadurch bedingten relativ hohen Blut-
druck in dem Filtrationsabschnitt eine grosse Menge Blutfiltrat gebildet, dem in
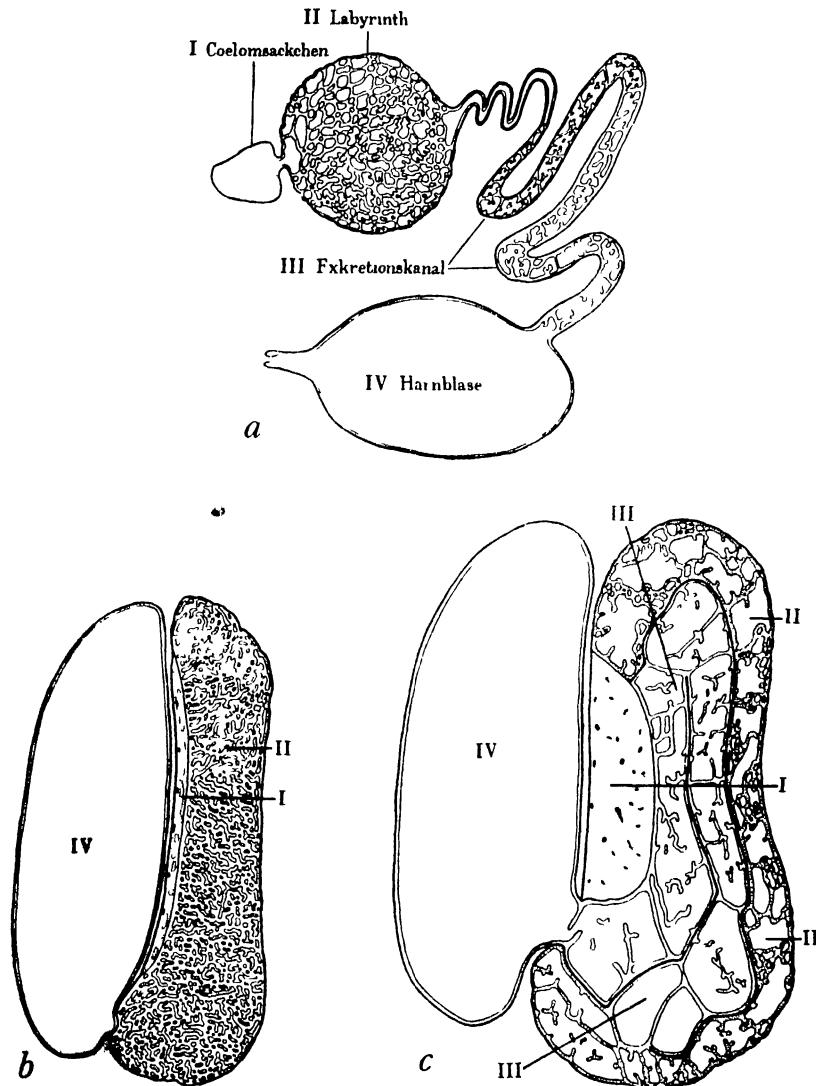
Abb. 3. (*a*) Schema eines Exkretionsorganes (Antennendruse) von *Potamobius fluviatilis* (nach
Marchal, 1892, verändert), (*b*) Schnitt durch ein Exkretionsorgan von *Homarus vulgaris* (Original
von H. Peters); (*c*) Schnitt durch ein Exkretionsorgan von *Potamobius fluviatilis* (Original von
H. Peters). I = Filtrationsabschnitt, II = Sekretionsabschnitt, III = Rückresorptionsabschnitt,
IV = Harnblase.

dem Sekretionsabschnitt geringe Mengen N-haltiger Exkrete beigefügt werden. In dem darauffolgenden Abschnitt, dem Harnkanälchen, werden aus dem salzreichen blutisotonischen, primären Harn die für den süsswasserlebenden Flusskrebs sehr wertvollen Salze zum grössten Teil zurückresorbiert[1]. Bei dem Hummer ist dagegen die für die Harnbildung zur Verfügung stehende Wassermenge bedeutend geringer, da das Innenmedium des Hummers nur ganz schwach hypertonisch gegenüber dem Aussenmedium ist. Es strömen also beim Hummer nur geringe Mengen Blutfiltrat aus dem Coelomsäckchen in die darauffolgenden Nierenteile. Um nun trotz dieser geringen Durchströmung der Nieren die gebildeten N-haltigen Exkrete vollständig aus dem Körper nach aussen schaffen zu können, ist der Sekretionsabschnitt der Nieren ausserordentlich viel grösser als beim Flusskrebs. Der auf den Sekretionsabschnitt folgende, beim Flusskrebs gut ausgebildete Rückresorptionsabschnitt fehlt vollständig, da eine Rückresorption von Salzen bei dem marinen Hummer nicht notwendig ist.

Auch bei den Nieren der *wasserlebenden Wirbeltiere* lässt sich die relative Entwicklung der filtratorischen und sekretorischen Abschnitte der Nieren in Beziehung zur Wasserausscheidung bringen. Bei den Nieren der süsswasserlebenden Dipnoer, Ganoiden, Teleostier und Amphibien sind die Filtrationsabschnitte (die Glomeruli) im Verhältnis zu den Sekretionsabschnitten (den Tubuli) stark entwickelt, die Nieren besitzen zahlreiche Glomeruli. Es werden grosse Mengen eines sehr verdünnten Harnes gebildet. Im Gegensatz dazu überwiegen bei den marinen Teleostiern in Anpassung an die geringen (durch Trinken aufgenommenen) zur Harnbildung zur Verfügung stehenden Wassermengen die Sekretionsabschnitte, denn die Glomeruli sind zahlenmässig und der Grösse nach zurückgebildet. Eine grosse Anzahl stenohaliner mariner Teleostier haben sogar aglomerulare Nieren. Die ausgeschiedenen Harnmengen sind dementsprechend sehr klein (Marshall und Smith, 1930).

Die Untersuchung des Harnes der Wassertiere bestätigt die auf Grund der Morphologie der Nieren gewonnenen Anschauungen und gibt uns weiteren Aufschluss über die osmoregulatorische Bedeutung dieser Organe (siehe Tabelle IV). Der Harn einiger homoiosmotischer Evertebraten—*Carcinus, Eriocheir, Telphusa*—ist blutisotonisch und enthält grosse Mengen anorganischer Salze (Schlieper, 1930; Scholles, 1933; Nagel, 1934). Diese Tatsache zwingt uns zu dem Schluss, dass die Osmoregulation bei diesen Arten ohne wesentliche Mithilfe der Exkretionsorgane zustande kommt, denn es ist undenkbar, dass bei einem Süsswassertier ein nennenswerter osmotischer Wassereinstrom durch Ausscheidung eines blutisotonischen, salzreichen Harnes ausgeglichen wird. Ein derartiger Mechanismus würde in kürzester Zeit den Verlust des gesamten lebensnotwendigen Salzvorrates herbeiführen. Die Nieren anderer Süsswasserevertebraten (*Potamobius*), die der süsswasserlebenden Teleostier und der Amphibien leisten dagegen osmoregulatorische Arbeit, indem sie grosse Mengen eines zum Blute hypotonischen, salzarmen Harnes produzieren und so das osmotisch aufgenommene Wasser ohne

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[1] In noch unveröffentlichten Untersuchungen konnte H. Peters im Zoologischen Institut Marburg, durch Mikroentnahmen und Mikroanalysen die Richtigkeit dieser Theorie beweisen.

Tabelle IV. *Die osmotischen Leistungen der Exkretionsorgane der Wassertiere*
(*Mittelwerte oder Beispiele nach Bottazzi, H. W. Smith, Schlieper und Scholles*).

Art	Aussen-medium Δ° C.	Harn Δ° C.	Blut Δ° C.
Marine Evertebraten			
Carcinus maenas	1,85	1,90	1,90
Carcinus maenas	1,00	1,50	1,50
Eriocheir sinensis	2,20	2,14	2,10
Homarus vulgaris	1,85	1,89	1,89
Susswasserevertebraten			
Potamobius fluviatilis	0,02	0,09	0,80
Eriocheir sinensis	0,02	1,23	1,20
Marine Elasmobranchier	1,85	1,92	1,93
Susswasserelasmobranchier	0,02	0,10	1,00
Susswasserteleostier	0,02	0,10	0,70
Marine Teleostier	1,85	0,70	0,80
Amphibien (Frosch)	0,02	0,17	0,44

zu grosse Salzverluste wieder hinausschaffen. Diese Beobachtungen erklären uns die oben erwähnten Unterschiede in der morphologischen Ausbildung der Exkretionsorgane bei den marinen und den süsswasserlebenden Evertebraten. Sie machen es uns aber auch verständlich, warum Ausnahmen von dieser Regel bestehen können, denn die einfachen durch das Fehlen eines längeren Harnkanales ausgezeichneten Exkretionsorgane der marinen Crustaceen liefern ebenso wie die ähnlich gebauten Organe der süsswasserlebenden Krabbe *Telphusa* einen blutisotonischen Harn. Dieser blutisotonische Harn, der bei *Homarus* und *Telphusa* direkt ausgeschieden wird, wird bei *Potamobius* durch Rückresorption in dem langen Harnkanal von dem grössten Teil seiner Salze befreit (Schlieper und Herrmann, 1930; Peters, 1935). Nur durch diese Rückresorptionsarbeit, nicht aber durch eine einfache Steigerung der Harnmenge—wie neuerdings in Übereinstimmung mit Hesse (1924) von Remane (1934) wieder angenommen wird—lassen sich die längeren Harnkanälchen der süsswasserlebenden Evertebraten erklären.

Der *Harn der Süsswasserhaie* hat eine ähnliche niedrige Molarkonzentration wie der der Süsswasserteleostier. Dagegen produzieren die marinen Elasmobranchier einen Harn, der isotonisch oder nur schwach hypotonisch gegenüber dem Blute ist. Die Regulierung des Wasser- und Mineralhaushaltes scheint trotzdem bei den süsswasserlebenden und den marinen Formen prinzipiell die gleiche zu sein. Die Ursache des hohen Harnstoffgehaltes im Blute ist wahrscheinlich einerseits die Tätigkeit der Nieren (durch die weniger Harnstoff ausgeschieden wird als sich im Körper bildet) und andererseits eine sehr geringe Durchlässigkeit der Aussenmembranen für Harnstoff. Der hohe Harnstoffgehalt des Blutes ermöglicht es den marinen Elasmobranchiern, den Salzgehalt des Innenmediums ohne grosse osmotische Arbeitsleistungen niedrig zu halten. Der Hauptunterschied zwischen den süsswasserlebenden und den marinen Formen besteht anscheinend darin, dass

die Nieren der marinen Arten in stärkerem Masse Harnstoff zurückhalten, und dass sie in grossem Masse Salze auf einem—später zu besprechenden—extrarenalen Wege ausscheiden (H. W. Smith, 1931).

Die Nieren der *marinen Teleostier* produzieren in geringen Mengen einen Harn, der im Verhältnis zum Blute iso- bezw. schwach hypotonisch ist. Eine Osmoregulation kann auf diese Weise nicht zustande kommen, sie vollzieht sich auf extrarenalem Wege, den wir in Kapitel V besprechen werden.

IV. OSMOREGULATORISCHE LEISTUNGEN DES DARMKANALES.

Bei einigen Wassertieren hat man eine osmoregulatorische Funktion des Darmkanales nachgewiesen. Beadle (1931, 1934) konnte bei der Triklade *Gunda ulvae* zeigen, dass das bei Brackwasserindividuen osmotisch durch die Haut eingedrungene Wasser von den Darmzellen in Form von intrazellulären Vakuolen aufgenommen und unschädlich gemacht wird. Bei Individuen, deren Atmung aber durch Zusatz von KCN zum Brackwasser herabgesetzt war, verschwanden die Vakuolen in den Darmzellen, während Ectoderm und Parenchym anschwollen. Es scheint danach die Bildung der Vakuolen in den Darmzellen eine mit einem Energieverbrauch verbundene Arbeitsleistung zu sein. Eine Ausscheidung des Vakuoleninhaltes in das Darmlumen war nie zu beobachten. Beadle möchte deshalb annehmen, dass bei *Gunda* ein osmotischer Wassereinstrom nur in der ersten Zeit nach der Überführung in Brackwasser besteht, der dann aber durch eine "passive" Abnahme der Wasserpermeabilität der Aussenmembranen verhindert wird. Eine ähnliche, noch wirksamere osmoregulatorische Leistung der Darmzellen beobachtete Harnisch (1934) bei den Larven von *Chironomus thummi*. Bei diesen in Süsswasser lebenden Insektenlarven wird das osmotisch von aussen eindringende Wasser von dem Enddarm ("Dickdarm") in Form von basalen "Stauvakuolen" aufgenommen, dann diffus distalwärts transportiert und unter Vermittlung von Säumen kleiner distaler "Leerungsvakuolen" in das Darmlumen ausgeschieden[1]. Die auf diese Weise bei einem Turbellar und einem Arthropoden nachgewiesene osmoregulatorische Leistung des Darmkanales legt den Gedanken nahe, dass ähnliche osmoregulatorische Mechanismen auch noch bei anderen Wassertieren vorhanden sind. Weitere Untersuchungen in dieser Richtung sind sehr erwünscht.

V. PERMEABILITÄT UND KONZENTRATIONSLEISTUNGEN DER KÖRPEROBERFLÄCHEN.

Trennt eine Membran zwei Medien von verschiedener Molarkonzentration, so tritt ein Ausgleich um so langsamer ein, je weniger durchlässig die betreffende Membran ist. Deshalb ist die Untersuchung der Permeabilität der Körperoberflächen der Wassertiere wichtig für die Beurteilung ihrer osmoregulatorischen Leistungen. Vernachlässigt man diese Forderung, so kann es vorkommen, dass eine

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[1] Auch Wigglesworth (1933 *a*, *b* u. *c*) beobachtete eine rhythmische Wasserabgabe aus dem Rectum bei Mosquitolarven. Er nimmt jedoch auf Grund seiner Beobachtungen im Gegensatz zu Harnisch an, dass die in den Darm mundenden Malpighischen Gefässe die eigentlichen Organe der Wasserausscheidung sind.

sehr geringe Permeabilität der Membranen das Vorhandensein einer osmotischen Resistenz oder eines osmoregulatorischen Mechanismus vortäuscht.

Die *Körperoberflächen der wasserlebenden Evertebraten* sind allgemein für *Wasser* durchlässig, nur der Grad der Wasserdurchlässigkeit weist grosse Unterschiede auf. Die Haut der stenohalinen Meeresevertebraten ist in gleichem Masse von innen nach aussen wie auch von aussen nach innen für Wasser durchlässig, ohne dass für den Organismus die Möglichkeit zu einer Regulation besteht. Die von Dekhuyzen (1920) bei *Phascolosoma* gefundene Resistenz gegenüber dem osmotischen Wassereinstrom bei Hypotonie des Aussenmediums lässt sich einfach auf Grund der Elastizität der Körperoberflächen erklären. Evertebraten, die in konzentriertem Meerwasser geschrumpft sind, nehmen in normalem Meerwasser mit der gleichen Geschwindigkeit Wasser auf, wie sie es in hypertonischen Medien abgeben (Koizumi, 1932). Dagegen ist wahrscheinlich bei zahlreichen homoiosmotischen Wassertieren die Wasserdurchlässigkeit der Aussenmembranen veränderlich. So steht z. B. der Grad der Wasserdurchlässigkeit der Kiemen des an Meerwasser angepassten Aales (*Anguilla vulgaris*) in direkter Beziehung zur Konzentration des Innenmediums. Die Kiemen derartiger Fische sind nämlich normalerweise für Wasser in der Richtung von innen nach aussen nur wenig durchlässig. Sie werden jedoch in grösserem Masse durchlässig, wenn man im Perfusionsexperiment die Konzentration des Innenmediums von $\Delta = 0{,}70°$ C. auf $\Delta = 0{,}50°$ C. herabsetzt[1] (Schlieper, 1933 b). Beim Frosch wird nach Adolph (1933) die Wasserdurchlässigkeit der Haut vom Nervensystem beeinflusst.

Die *Aussenmembranen der marinen Evertebraten* sind in beiden Richtungen für *Salze* durchlässig (Bethe, 1929; Dakin und Edmonds, 1931; Koizumi, 1932). Da aber bei vielen marinen Evertebraten Wasser schneller permeiert als Salze, ist die Durchlässigkeit für letztere oft übersehen worden. Führt man ein weichhäutiges, stenohalines Meeresevertebrat in Brackwasser über, so diffundieren wohl auch Salze in kleinen Mengen durch die Körperoberflächen nach aussen, aber die relativ grössere Wasserdurchlässigkeit bewirkt einen so starken osmotischen Wassereinstrom, dass die Exkretionsorgane dem nicht standhalten können und eine rapide Gewichtszunahme des ganzen Tieres die Folge ist. Erst nachdem zwischen Innen- und Aussenmedium ein Ausgleich eingetreten ist, wird diese Gewichtszunahme im Laufe der darauffolgenden Stunden wieder rückgängig gemacht (Bethe, 1930; Hukuda, 1932). Diese Wiederherstellung des normalen Gewichtes nach starker osmotischer Wasseraufnahme hat mit osmoregulatorischen Vorgängen nichts gemein, es handelt sich hier wahrscheinlich um ein einfaches Hinauspressen des zuviel im Organismus vorhandenen Wassers. Andere harthäutige, euryhaline Arten (z. B. *Carcinus maenas*) passen sich nach Überführung in Brackwasser in der

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[1] $\Delta = 0{,}70°$ C. entspricht annähernd der Molarkonzentration des Blutes des seewasserlebenden Aales. Dadurch dass bei einer derartigen Konzentration des Blutes die Aalkiemen in der Richtung von innen nach aussen nur sehr wenig für Wasser durchlässig sind, ist beim Seewasseraal der durch die Hypertonie des Aussenmediums bedingte schädliche osmotische Wasserentzug auf ein Minimum herabgedrückt. Für den süsswasserlebenden Aal, dessen Blut eine niedrigere Konzentration aufweist, ist jedoch eine grössere Wasserpermeabilität der Kiemen in der Richtung von innen nach aussen unschädlich, da bei ihm das Aussenmedium ja hypotonisch gegenüber dem Innenmedium ist.

Hauptsache durch Abgabe von Salzen durch die Körperoberflächen hindurch an das neue Aussenmedium an. Es wäre aber falsch, nun aus diesem Befund auf eine Wasserimpermeabilität der Aussenmembranen dieser Tiere schliessen zu wollen, denn in Wirklichkeit findet auch bei diesen Formen in Brackwasser ein osmotischer Wassereinstrom statt. Die Exkretionsorgane sind hier aber in der Lage durch vermehrte Harnausscheidung eine Gewichtszunahme des Tieres zu verhindern. Einwandfrei lässt sich die Salzpermeabilität der Körperoberflächen der marinen Evertebraten auf folgende Weise zeigen. Man führt ein Exemplar einer weichhäutigen Art (z. B. *Aplysia punctata*) in ein Gemisch von Seewasser und isotonischer Rohrzuckerlösung über. Die Molarkonzentration ist bei diesem Experiment im Innen- und Aussenmedium gleich, nur die Salzkonzentration ist aussen geringer. Als Folge davon diffundieren Salze von innen nach aussen, während der grossmolekulare Rohrzucker nicht mit der gleichen Geschwindigkeit permeieren kann. Dadurch sinkt die Molarkonzentration im Inneren; das Versuchstier gibt zum Ausgleich osmotisch Wasser ab und sein Gewicht sinkt dementsprechend (Bethe, 1930). Sehr einfach demonstriert sich die Salzpermeabilität der Aussenmembranen auch, wenn man marine Evertebraten (*Aplysia*, *Caudina*) in künstliches Seewasser von wenig veränderter Zusammensetzung überführt und die Wirkung auf die Ionenrelation im Innenmedium untersucht (Bethe, 1929). Man findet dann stets—auch wenn man physiologisch-äquilibrierte, isotonische Lösungen verwendet—nach einiger Zeit eine weitgehende Angleichung der ionalen Zusammensetzung der Körpersäfte an das benutzte Aussenmedium. Dieser Ausgleich tritt auch dann ein, wenn eine Mitwirkung des Darmtractus ausgeschaltet ist. Die Geschwindigkeit des Ionenaustausches durch die Körperoberflächen der Holothurie *Caudina chilensis* erfolgte in der Reihenfolge K > Na > Ca > Mg; Cl > SO_4 (Koizumi, 1932). Das ist die lyotrope Reihe, die uns durch Permeabilitätsuntersuchungen an zahlreichen Organismen bekannt geworden ist.

Auch die *Aussenmembranen der Süsswasserevertebraten und der Fische* sind nicht semipermeabel. Zahlreiche Beobachtungen weisen daraufhin, dass zumindest eine einseitige Salzpermeabilität besteht. Sämtliche Süsswassertiere nehmen z. B. Salze, die dem Aussenmedium in kleinen Mengen zugefügt worden sind (z. B. KCl oder NaJ), durch Diffusion und zusammen mit dem osmotisch eindringenden Wasser auf. Sehr wahrscheinlich ist es aber, dass die Körperoberflächen der Süsswassertiere in der Richtung von innen nach aussen für Salze annähernd impermeabel sind. Ebenso müssen wir für die Oberflächen der marinen Teleostier und Elasmobranchier eine ausserordentlich geringe Permeabilität für Salze in der Richtung von aussen nach innen annehmen. Wäre dies nicht der Fall, so wäre eine Osmoregulation bei diesen Tieren nur unter sehr grossem Energieverbrauch denkbar. Aber auch hier hat es den Anschein, als ob es sich nicht um eine konstante einseitige Impermeabilität für Salze handelte, sondern um einen physiologischen Zustand der Membranen (eine elektrische Ladung), der leicht durch irgendwelche Eingriffe verändert werden kann. Erhöht man z. B. durch Injektion oder auf eine andere Weise die Salzkonzentration des Innenmediums bei einem Süsswasserwirbellosen, so werden seine Membranen für Salze solange in der Richtung von innen nach aussen durch-

lässig, bis durch Diffusion der zuviel im Inneren vorhandenen Salze nach aussen die ursprüngliche Differenz zwischen der Zusammensetzung des Innen- und Aussenmediums wiederhergestellt worden ist (Berger, 1931).

Allgemein scheint die Durchlässigkeit der Aussenmembranen euryhaliner Wassertiere geringer zu sein als die ihrer stenohalinen Verwandten. Vergleicht man die Anpassungsgeschwindigkeiten mariner Evertebraten an hypotonische Medien, so findet man stets, dass sie bei den euryhalinen Arten am kleinsten ist. Während z. B. die Zeit bis zur Ausbildung eines neuen Gleichgewichtszustandes zwischen Innen- und Aussenmedium bei dem stenohalinen Krebs *Maja verrucosa* nur wenige Stunden beträgt, braucht der euryhaline *Carcinus maenas* einen halben bis einen ganzen Tag. Die extrem euryhaline Wollhandkrabbe *Eriocheir sinensis* benötigt nach der Überführung aus Meer- in Süsswasser und umgekehrt sogar ein bis zwei Wochen (Schlieper). Es ist nun nicht einfach, klar zu beweisen, dass diese geringe Anpassungsgeschwindigkeit bei den euryhalinen Arten durch eine geringe Wasserpermeabilität der Aussenmembranen begünstigt wird, denn wir kennen tatsächlich noch keine Methode, welche die Wasserpermeabilität der Oberflächen dieser Tiere allein— ohne Auftreten von Salzdiffusionen, Gegenregulationen usw.—zu messen gestattet (Bethe, 1934). Trotzdem möchte ich annehmen, dass die Gewichtsänderungen, die marine Evertebraten in hypotonischen Medien durch Endosmose erleiden, uns zumindest ein gewisses Bild von der Grössenordnung der Wasserpermeabilität ihrer Oberflächen liefern, solange man nur die Gewichtsmessungen während der ersten Stunden nach der Überführung in das hypotonische Medium verwertet. Derartige Beobachtungen zeigen aber tatsächlich, dass stenohaline Arten im Vergleich zu euryhalinen sehr schnell Wasser unter den genannten Versuchsbedingungen aufnehmen. Ganz einwandfrei lässt sich aber beweisen, dass die Aussenmembranen stenohaliner poikilosmotischer Meeresevertebraten leichter Salze in beiden Richtungen durchlassen, als die ihrer euryhalinen, fakultativ homoiosmotischen und süsswasserlebenden Verwandten (Schlieper, 1932; Nagel, 1934) (siehe Abb. 4).

Verschiedene Beobachtungen machen es wahrscheinlich, dass auch bei den Fischen der Permeabilitätsgrad der Haut ausschlaggebend ist für ihre osmoregulatorischen Leistungen. Seit langem ist es z. B. bekannt, dass der Aal (*Anguilla vulgaris*) und andere euryhaline Teleostier ohne die ihre Haut bedeckende, wenig durchlässige Schleimhülle nicht mehr imstande sind, plötzliche grosse Änderungen in der Salzkonzentration des Aussenmediums zu ertragen (siehe u. a. Duval, 1925; Firly, 1932). Wir erkennen aus diesen Beobachtungen die Bedeutung der Permeabilität der Körperoberflächen für die Osmoregulation der Wassertiere. Wir müssen uns aber auch darüber klar sein, dass auf Grund einer niedrigen Permeabilität der Aussenmembranen allein niemals auf die Dauer eine Homoiosmie zustande kommen kann, denn es handelt sich hierbei—wie gesagt—ja nicht um einen konstanten Zustand sondern um ein dynamisches Gleichgewicht.

Eine neuere Arbeitshypothese besagt, dass die Körperoberflächen zahlreicher Wassertiere die Fähigkite besitzen, Wasser und Salze je nach den Erfordernissen des Organismus von aussen nach innen bezw. von innen nach aussen zu transportieren. In einigen Fällen ist es gelungen, die Beteiligung derartiger Vorgänge

an der Osmoregulation wasserlebender Tiere wahrscheinlich zu machen. Von wirbellosen Tieren ist *Carcinus maenas* in dieser Richtung am eingehendsten untersucht worden. Diese in Brackwasser homoiosmotische Krabbe scheidet—wie schon oben erwähnt—unabhängig von der Konzentration des Aussenmediums stets einen blutisotonischen Harn aus. Die Exkretionsorgane können also nicht die Ursache der osmoregulatorischen Leistungen von *Carcinus* sein, im Gegenteil, der Krebs verliert im Brackwasser durch eine entsprechend dem osmotischen Wassereinstrom erhöhte Harnausscheidung ständig grosse Salzmengen. Die Körperoberflächen—d. h. die Kiemen—gewährleisten wahrscheinlich die Hypertonie des Blutes dadurch, dass sie einerseits vermöge ihrer geringen Permeabilität den osmotischen Ausgleich
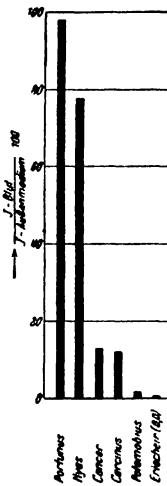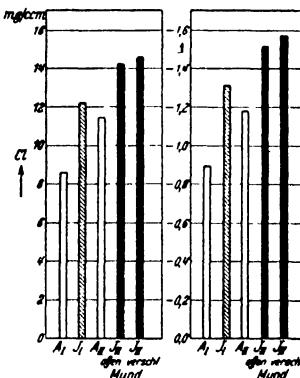
Abb. 4.   Abb. 5.

Abb. 4. Vergleich der Permeabilität der Körperoberflächen verschiedener dekapoder Crustaceen. Jodidgehalt des Blutes nach 2,5-stündigem Aufenthalt in NaJ-haltigem Aussenmedium (nach Nagel).
Abb. 5. Nachweis einer Salzaufnahme durch die Kiemen von *Carcinus maenas*. A = Aussenmedium, J = Innenmedium (nach Nagel).

verzögern und andererseits Salze aus dem Aussenmedium nach innen transportieren. Den Nachweis einer derartigen aktiven Salzaufnahme durch die Kiemen glaubt Nagel (1934), auf folgende Weise, erbracht zu haben. Eine Anzahl Exemplare aus Brackwasser (Aussenmedium Nr. 1) wurden in ein Medium von etwas höherem Salzgehalt (Aussenmedium Nr. 2) gebracht, das aber dem ursprünglichen Blut (Innenmedium Nr. 1) gegenüber noch hypotonisch war. Nach 24 Stunden ergab dann die Analyse des Blutes der Krebse (Innenmedium Nr. 2) einen Anstieg der Konzentration im Inneren (siehe Abb. 5). Die Krebse haben also Salze aufgenommen, obwohl in ihrem Blut während der ganzen Dauer des Versuches eine höhere Salz- und Molarkonzentration herrschte als im Aussenmedium. Da das Ergebnis des Versuches das gleiche ist, wenn der Mund der Krebse verschlossen ist, so dürfen wir annehmen, dass die Kiemenzellen der Krebse die Salze entgegen dem osmotischen

Gefälle nach innen transportiert haben[1]. Durch diese Fähigkeit der Kiemen von *Carcinus* erklärt sich die Hypertonie des Blutes der Brackwasserindividuen. Die Kiemen transportieren unter diesen Umständen, so kann man annehmen, dauernd soviel Salze von aussen nach innen, wie dem Krebs durch andere Prozesse (Harnausscheidung usw.) verloren gehen (Nagel, 1934). Es ist denkbar, dass diese Fähigkeit Salze von aussen durch die Haut aktiv aufzunehmen nicht nur bei den euryhalinen Brackwasserevertebraten sondern auch bei den Süsswassertieren weit verbreitet ist, und dass sich manche noch ungeklärte Prozesse, wie z. B. die Kalkaufnahme der Crustaceen und Lamellibranchier, vielleicht auf einen ähnlichen Mechanismus zurückführen lassen werden.

Bei den *marinen Teleostiern* leisten die Kiemen die hauptsächliche osmoregulatorische Arbeit, denn die Nieren der Knochenfische sind nicht imstande, einen im Verhältnis zum Blute hypertonischen Harn zu bilden. Eine erste Vorstellung davon, auf welche Weise sich die marinen Teleostier das zur Harnbereitung notwendige Wasser verschaffen und wie bei ihnen die Osmoregulation zustande kommt, lieferten die Untersuchungen von H. W. Smith (1930). Dieser Autor zeigte, dass die marinen Knochenfische regelmässig Seewasser trinken und den grössten Teil dieses Wassers, sowie die darin enthaltenen Na-, K- und Cl-Ionen auch resorbieren, jedoch ohne dabei anscheinend irgendwelche osmotische Arbeit zu leisten. Da aber von seiten des Darmkanals bedeutend mehr K, Na und Cl aufgenommen werden, als im Anschluss daran durch die Nieren ausgeschieden wird, muss der restliche Teil der Salze auf irgendeinem anderen Wege den Körper verlassen. Es liess sich beweisen, dass der Darm und die Haut als Ort dieser extrarenalen Ausscheidung nicht in Frage kommen. Smith nahm deshalb an, dass es die Kiemen sind, welche den Überschuss an Salzen in Form einer im Verhältnis zum Seewasser hypertonischen Lösung ausscheiden und so den Nieren die Bildung eines salzarmen Harnes ermöglichen. Keys (1931 b) erbrachte den Beweis für die Richtigkeit dieser Annahme. Er untersuchte mit Hilfe von Perfusionsexperimenten die Tätigkeit der Kiemen des an Meerwasser angepassten Aales (*Anguilla vulgaris*) und fand, dass die Kiemenzellen dieses Fisches in Meerwasser eine mehr oder weniger konzentrierte Chloridlösung in der Richtung von innen nach aussen transportieren. Je höher der Chloridgehalt des als Innenmedium benutzten Perfusates war, um so stärker war auch die beobachtete Chloridausscheidung. Ich selbst (Schlieper, 1933 a) konnte im Anschluss hieran zeigen, dass diese Chloridausscheidung der Kiemen des an Meerwasser angepassten Aales eine spezifische Chloridregulation der Körpersäfte darstellt, die durch einen hohen Chloridgehalt der Kiemenzellen selbst ausgelöst wird und rein aktiv erfolgt, ohne dass einfache physikalische Vorgänge—etwa nach Art der Einstellung eines Donnan-Gleichgewichtes—daran mitbeteiligt sind. Auch bei Isotonie des Aussenmediums transportieren die Kiemen des Aales, so fand ich, Chloride von innen nach aussen, sofern nur der Chloridgehalt der Kiemenzellen gegenüber dem der Kiemen des Süsswasseraales erhöht ist. Damit wäre das Problem der Osmoregulation der marinen Teleostier zu einem wesentlichen Teil gelöst.

[1] Eine weitere experimentelle Prüfung dieser Arbeitshypothese erscheint mir erwünscht.

Untersuchungen von H. W. Smith (1931) machen es wahrscheinlich, dass ähnliche extrarenale Ausscheidungen von Salzen, wie wir sie bei den marinen Teleostiern kennen gelernt haben, auch bei den marinen Elasmobranchiern vorkommen.

VI. ATMUNG UND OSMOREGULATION (OSMOREGULATORISCHER ENERGIEVERBRAUCH).

Die Osmoregulation der Wassertiere ist notwendigerweise ebenso wie jede andere Arbeitsleistung des lebenden Organismus mit einem Energieverbrauch verbunden. Dieser Energieverbrauch wird um so grösser sein je höher die Differenz zwischen den Konzentrationen des Innen- und Aussenmediums ist und je durchlässiger die Körperoberflächen sind. Exakte Messungen und Berechnungen des osmoregulatorischen Energieverbrauches eines Wassertieres existieren noch nicht. Versuche, wie sie in dieser Richtung von Graetz (1931) für den Süsswasserstichling (*Gasterosteus aculeatus*) und von Keys (1931 b) für den an Meerwasser angepassten Aal (*Anguilla vulgaris*) unternommen worden sind, haben infolge des Mangels an einwandfreien Unterlagen nur sehr hypotethische Bedeutung. In vielen Fällen sind wir uns über die Art der Energiequellen—ob Oxydationen oder anoxydative Prozesse—noch nicht im Klaren. Auf Grund unseres augenblicklichen Wissens dürfen wir jedenfalls annehmen, dass die folgenden osmoregulatorischen Vorgänge mit einem Energieverbrauch verknüpft sind: (1) Die Bildung eines zum Blute hypotonischen Harnes (z. B. bei den Süsswasserteleostiern) und (2) ein Transport von Salzen durch die Körperoberflächen entgegen dem osmotischen Gefälle (z. B. die Chloridausscheidung der Kiemen der marinen Teleostier). Fraglich ist es noch, ob die Aufrechterhaltung einer einseitigen Permeabilität (z. B. bei den Körperoberflächen der Süsswassertiere) eine Arbeitsleistung darstellt, dafür spricht vielleicht, dass die Irreprozität und Potentialdifferenz der Froschhaut beim Absterben abnehmen (Krijgsman, 1932). Dagegen kommt die Produktion eines zum Blute isotonischen Harnes wahrscheinlich auf Grund eines einfachen Filtrationsprozesses in den Exkretionsorganen ohne nennenswerten Energieverbrauch zustande (z. B. bei *Carcinus*). Um diese Fragen zu entscheiden, sind vor allem Bestimmungen des Sauerstoffverbrauches usw. isolierter osmoregulatorischer Organe im Perfusionsexperiment erwünscht, Untersuchungen, die leider noch gänzlich fehlen. Dagegen hat man schon den Versuch gemacht, den Sauerstoffverbrauch nahe verwandter mariner und süsswasserlebender Evertebraten unter den gleichen Bedingungen zu messen. Fox und Simmonds (1933) haben hierbei gefunden, dass der Sauerstoffverbrauch der süsswasserlebenden Crustaceen *Gammarus pulex* und *Asellus aquaticus* ungefähr zwei- bis dreimal so gross ist, wie der ihrer marinen Verwandten *Gammarus locusta* und *Idotea neglecta*. Man kann diese Unterschiede in der Energieproduktion auf Grund artverschiedener Stoffwechselintensitäten erklären, begründeter erscheint aber wohl die Hypothese, welche für den höheren Sauerstoffverbrauch der Süsswasserarten ihre Osmoregulation (insbesondere die Bildung eines gegenüber dem Blute hypotonischen Harnes) verantwortlich macht. Für die Richtigkeit dieser

letzteren Deutung spricht auch die Tatsache, dass gewisse Süsswassertiere (*Paramaecium, Potamobius*), deren Exkretionsorgane bei Isotonie des Aussenmediums sistieren, in isotonischem Seewasser, eine beträchtlich herabgesetzte Atmung haben (Hayes, 1930; Schwabe, 1933).

Auch bei marinen Wirbellosen hat man den Sauerstoffverbrauch bei Isotonie und Anisotonie des Aussenmediums gemessen. Schlieper (1929, 1931) hat bei verschiedenen euryhalinen marinen Evertebraten (*Carcinus maenas, Gammarus locusta, Nereis diversicolor*) in Brackwasser eine starke Steigerung des Sauerstoffverbrauches gefunden[1]. Man kann die Ursache dieser Erscheinung nicht in einer gesteigerten Leistung der Exkretionsorgane suchen, weil auch isolierte Kiemenstücke von *Mytilus edulis* in Brackwasser die gleiche Atmungssteigerung zeigen. Schlieper stellte deshalb die Hypothese auf, dass die Erhöhung des Sauerstoffverbrauches durch eine von den Körperoberflächen gegen den Wassereinstrom von aussen wirkende Arbeitsleistung (aktive osmotische Resistenz) verursacht werde. Versuche von Beadle (1931) scheinen die Richtigkeit dieser Ansicht zu bestätigen, denn sie zeigten, dass *Nereis diversicolor* und *Gunda (Procerodes) ulvae* unter der atmungshemmenden Wirkung von KCN sowie in sauerstofffreien Medien die Hypertonie ihrer Körpersäfte in Brackwasser nicht aufrechterhalten. Auch Schwabe (1933) fand, dass *Carcinus* in Brackwasser bei Verhinderung der Atmungssteigerung durch Verminderung des Sauerstoffdruckes im Aussenmedium nicht mehr imstande ist, seine normale Hypertonie zu bewahren. Diese Befunde scheinen tatsächlich dafür zu sprechen, dass die in Brackwasser erhöhte Energieproduktion mit der Osmoregulation der Evertebraten in diesen Medien in Zusammenhang steht[2]. Eine gewisse Schwierigkeit bietet allerdings das Verhalten von *Nereis diversicolor*. Schlieper und Beadle beobachteten nämlich beide, dass *Nereis* nach Überführung in Brackwasser nur während der ersten Stunden eine Erhöhung des Sauerstoffverbrauches aufweist, obgleich dieser Polychaet in Brackwasser dauernd homoiosmotisch ist. Beide Autoren brachten diese Erscheinung in Verbindung mit der nach Überführung in Brackwasser durch osmotischen Wassereinstrom bewirkten vorübergehenden Gewichtserhöhung von *Nereis* und glaubten in der Annahme eine ausreichende Erklärung gefunden zu haben, dass bei *Nereis* eine aktive osmotische Resistenz und dementsprechend ein erhöhter Energieverbrauch nur während des vorübergehenden starken osmotischen Wassereinstromes existiere. Wir wissen heute ausserdem, dass gewisse andere euryhaline Evertebraten (*Eriocheir sinensis, Pelmatohydra oligactis, Clava multicornis*) sowohl bei Isotonie wie auch bei Hypotonie des Aussenmediums denselben Sauerstoffverbrauch haben (Schlieper, 1932; Palmhert, 1933; Schwabe, 1933). Weiterhin fand Schlieper (1931), dass euryhaline

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[1] Noch unveröffentlichte Experimente von O. Löwenstein aus dem Zoologischen Laboratorium der Universität Birmingham über die Atmungsintensität von *Gammarus chevreuxi* in Wasser von verschiedenen Salzgehalten haben ergeben, dass der Sauerstoffverbrauch dieses Brackwasseramphipoden in reinem Seewasser um 20 % geringer ist als in auf ein Viertel verdünntem Seewasser. Die Atmungsintensität von *G. chevreuxi* in einem derartigen Brackwasser, dessen Salzgehalt ungefähr dem seines Herkunftsortes entspricht, liegt zwischen den von Fox und Simmonds (1933) gefundenen Werten für *G. pulex* (Süsswasser) und *G. marinus* (Meer).

[2] Ich erinnere in diesem Zusammenhang an den Befund von Simon (1922), nach welchem durch Erstickung die Permeabilität der Muskelfasermembranen erhöht wird.

Evertebraten, die in Brackwasser einen erhöhten Sauerstoffverbrauch haben,
dieselbe Erscheinung auch in blutisotonischen Lösungen reiner Salze zeigen.
Brackwasser und blutisotonische Lösungen reiner Salze bewirken aber nicht nur
eine Atmungssteigerung sondern auch eine Hydratation (Quellung) der atmenden
Gewebe bei den Versuchstieren (Pieh, 1935). Diese Beobachtungen geben uns
Veranlassung, das Problem der Atmungssteigerung in Brackwasser erneut zur
Diskussion zu stellen und zunächst einmal die Bedeutung der Hydratation der
Gewebe in diesem Zusammenhang näher zu untersuchen. Betrachten wir unter
diesem Gesichtspunkt z. B. *Carcinus maenas*, so finden wir, dass der Gesamt-
wassergehalt dieses Krebses, gemessen am Körpergewicht, in Meer- und Brack-
wasser derselbe ist, dass aber die atmenden Gewebe bei Brackwasserindividuen einen
erhöhten Wassergehalt aufweisen. So ist z. B. der Wassergehalt der Kiemen von
Carcinus in Brackwasser von 20 $^{\circ}/_{\circ\circ}$ Salzgehalt ungefähr um 3 % und in 10 $^{\circ}/_{\circ\circ}$
Salzgehalt annähernd um 6 % im Vergleich zu dem Wassergehalt der Kiemen von
an reines Seewasser angepassten Krebsen erhöht. Blutisotonische NaCl-Lösung
bewirkt aber auch bei *Carcinus*—ebenso wie Brackwasser—eine Steigerung der
Atmung und eine Hydratation der Gewebe. Unterdrückt man die Atmungssteigerung
durch NaCN oder durch Verwendung sauerstoffreier Lösungen, so hat dies weder
einen fördernden noch einen hemmenden Einfluss auf die Hydratation der Gewebe.
Daraus kann man wohl schliessen, dass die Hydratation der primäre Vorgang ist,
durch den erst sekundär die Atmungssteigerung ausgelöst wird. Bei *Eriocheir
sinensis*, der in Meer- und Süsswasser denselben Sauerstoffverbrauch aufweist,
haben die Gewebe bei Meer- und Süsswasserindividuen den gleichen Hydratations-
grad (Pieh, 1935). Es bestehen demnach deutliche Beziehungen zwischen der
Atmungsintensität und dem Hydratationsgrad der Gewebe bei den untersuchten
Tieren. Ähnliches hat man aber schon früher bei landlebenden Organismen
beobachtet. So steigt z. B. bei *Helix pomatia* der Sauerstoffverbrauch mit zuneh-
mendem Wassergehalt der Zellen (Fischer und Duval, 1931). D. J. Lloyd (1932),
die in einem interessanten Referat zahlreiche ähnliche Angaben aus der Literatur
zusammengetragen hat, weist u. a. daraufhin, dass der Wassergehalt des Hühner-
embryos am 4. und 5. Tage—der Periode der grössten Aktivität in der Entwicklung
—am höchsten ist. Alle diese Beobachtungen geben uns Anlass zu der Vermutung,
dass die bei euryhalinen Meeresevertebraten aufgedeckten Beziehungen zwischen
Atmungsintensität und Hydratationsgrad der Gewebe in den Rahmen eines
allgemeingültigen biologischen Gesetzes fallen. Es ist nun noch die Frage zu
beantworten, welche Bedeutung der Faktor Osmoregulation in dem System Atmung
und Hydratation der Gewebe hat. Die Atmungserhöhung in Brackwasser ist nicht
die Folge der Osmoregulation in diesem Medium, das zeigen die berichteten
Ergebnisse einwandfrei. Man darf hieraus aber nicht schliessen, dass zwischen der
Atmungssteigerung in Brackwasser und der Osmoregulation keinerlei Beziehungen
bestehen. Das Gegenteil ist vielmehr der Fall, wie ebenso eindeutig die oben
erwähnten Versuche von Beadle und Schwabe beweisen. Die Atmungssteigerung
ist allerdings nicht die *Folge* der osmoregulatorischen Leistung, sondern—so
müssen wir nun annehmen—die *Voraussetzung* für das Funktionieren der osmo-

regulatorischen Mechanismen in Brackwasser. Bei *Eriocheir* besteht in Brackwasser infolge der geringen Durchlässigkeit der Körperoberflächen nur ein sehr schwacher osmotischer Wassereinstrom; der Hydratationsgrad der Gewebe bleibt deshalb unverändert, und die osmoregulatorischen Mechanismen sind leicht in der Lage, den an sie gestellten Anforderungen zu genügen. Anders verhält sich *Carcinus* in Brackwasser. Bei ihm besteht infolge der relativ grossen Durchlässigkeit der Körperoberflächen ein beträchtlicher osmotischer Wassereinstrom, der zu einer Hydratation der Gewebe führt, die ihrerseits eine Atmungssteigerung auslöst. Mit dieser Atmungssteigerung ist eine Herabsetzung der Permeabilität der Körperoberflächen verbunden, welche den osmotischen Wassereinstrom derart reduziert, dass die Leistungsfähigkeit der osmoregulatorischen Mechanismen ausreicht. Wird aber durch Verwendung sauerstoffarmen Brackwassers die Atmungssteigerung verhindert, so ist die Permeabilität der Aussenmembranen und damit der osmotische Wassereinstrom so gross, dass die osmoregulatorischen Mechanismen nicht mehr imstande sind, die normale Hypertonie des Innenmediums gegenüber dem Aussenmedium aufrechtzuerhalten.

Ausserordentlich kompliziert sind die Beziehungen zwischen Atmung und Salzgehalt des Aussenmediums bei den Teleostiern, weil diese Organismen ja nicht nur in Süsswasser sondern auch im Meer eine aktive Osmoregulation haben. Während Keys (1931 *a*) bei *Fundulus parvipinnis* in Meer- und Süsswasser denselben Sauerstoffverbrauch fand, beobachteten Raffy und Fontaine (1930) an jungen, eben in die Flüsse einwandernden Aalen (*Anguilla vulgaris*) eine beträchtliche Erhöhung der Atmung in Süsswasser.

VII. DIE BEDEUTUNG DER OSMOREGULATION FÜR DIE BIOLOGIE UND DIE ÖKOLOGIE DER WASSERTIERE.

Das Studium der osmoregulatorischen Eigenschaften der Wassertiere hat wesentlich zum Verständnis ihrer Biologie und Ökologie beigetragen. Während wir bisher die Begriffe *euryhalin* und *stenohalin* nur durch die Fähigkeit, grössere bezw. kleinere Schwankungen im Salzgehalt des Aussenmediums ertragen zu können definierten, wissen wir jetzt, dass die Ursache der Euryhalinie eines Wassertieres im Wesentlichen in dem Vorhandensein einer leistungsfähigen Osmoregulation zu suchen ist. Die Besiedlung des Brack- und Süsswassers vom Meere her ist nur durch die Ausbildung osmoregulatorischer Mechanismen möglich gewesen. Während zumindest die primären Bewohner des Meeres keinerlei osmoregulatorische Fähigkeiten besitzen, sind im Süsswasser nur homoiosmotische Organismen lebensfähig. Parallel mit der Entwicklung osmoregulatorischer Einrichtungen treten bei den marinen Einwanderern in das Süsswasser Veränderungen auf, welche die Osmoregulation in diesem Medium erleichtern, wie z. B. Herabsetzung der Permeabilität der Aussenmembranen (Nagel, 1934) und Bildung einer geringeren Anzahl relativ grosser dotterreicher Eier, von denen jedes mit einem beträchtlichen Salzvorrat ausgestattet ist (Needham, 1930). Manche Organismen, die erst in neuerer Zeit in das Süsswasser einwandern, haben noch unzureichende osmoregu-

latorische Mechanismen. Aus diesem Grunde muss z. B. *Eriocheir sinensis* alljährlich zum Ablaichen in das Meer zurückwandern. Während die Krabben ausserhalb der Fortpflanzungszeit in Süsswasser lebensfähig sind, können eiertragende Weibchen die normale Konzentration ihrer Körpersäfte in Süsswasser nicht aufrechterhalten (Scholles, 1933). Wesentlich für das Funktionieren der osmoregulatorischen Einrichtungen der Süsswassertiere erscheint ein gewisser Ca-Gehalt des Süsswassers. Sehr schön zeigte diese Tatsache Pantin (1931) an der Triklade *Gunda* (*Procerodes*) *ulvae*, welche Salzkonzentrationsschwankungen von Seewasser bis zu reinem Süsswasser vertragen kann. Pantin brachte *Gunda* aus Seewasser in Flusswasser und in destilliertes Wasser. Hierbei verhielt sich *Gunda* nur in dem Flusswasser homoiosmotisch, während sie in dem destillierten Wasser wie ein poikilosmotisches Evertebrat durch osmotische Wasseraufnahme anschwoll und gleichzeitig Salze abgab. Die genaue Analyse dieser Erscheinung führte zu der Erkenntnis, dass der geringe Ca-Gehalt des Flusswassers der Faktor war, der *Gunda* in diesem Medium lebensfähig machte. Da aus den Untersuchungen anderer Autoren bekannt ist, dass die Ca-Ionen die Permeabilität der tierischen Membranen herabsetzen, ist es wahrscheinlich, dass die Wirkung des Ca auch in dem genannten Flusswasser in einer Verringerung der Permeabilität der Oberflächen bei *Gunda* beruhte. Auch alteingesessene Süsswassertiere, wie z. B. *Potamobius fluviatilis* sterben in destilliertem Wasser—wahrscheinlich aus dem gleichen Grunde —innerhalb kurzer Zeit durch den Verlust ihres Salzvorrates (Huf, 1933).

Für die Besiedlung des Brackwassers durch marine Tiere gilt ähnliches wie für das Süsswasser. Da nur ein Teil der marinen Evertebraten eine starke Verdünnung der Körpersäfte verträgt, sind zahlreiche Arten allein vermöge ihrer osmoregulatorischen Fähigkeiten in Brackwasser lebensfähig. Auf Grund dieser Tatsache können z. B. der poikilosmotische *Asterias rubens* und der homoiosmotische *Carcinus maenas* nebeneinander in einem Brackwasser von 15 °/$_{oo}$ Salzgehalt existieren. *Asterias* deshalb weil dieser Salzgehalt noch die für seine Körpersäfte notwendige Minimalkonzentration übersteigt. Bei *Carcinus* hat zwar der Salzgehalt im Aussenmedium bereits die für die Körpersäfte erforderliche Minimalkonzentration unterschritten, der Krebs ist aber trotzdem lebensfähig, weil seine osmoregulatorischen Mechanismen die Konzentration seines Innenmediums hinreichend über der des umgebenden Brackwasser erhalten. Der plötzliche Absturz der Artenzahl mariner Formen in Brackwasser bei 10-8 °/$_{oo}$ Salzgehalt erklärt sich wahrscheinlich dadurch, dass die poikilosmotischen Formen eine stärkere Verdünnung ihrer Körpersäfte nicht ertragen, und dass dementsprechend in Brackwasser unter 10-8 °/$_{oo}$ Salzgehalt in der Hauptsache nur noch die relativ wenigen homoiosmotischen Formen lebensfähig sind.

Auch das Fehlen zahlreicher Süsswasserarten selbst in iso- oder hypotonischem Brackwasser lässt sich auf Grund unserer Kenntnisse von der Osmoregulation der Süsswassertiere erklären. Die Exkretionsorgane der meisten Süsswassertiere sind eben auf einen dauernden osmotischen Wassereinstrom durch die Haut eingestellt. Fehlt dieser Wassereinstrom in isotonischem Brackwasser oder ist er in hypotonischem Brackwasser vermindert, so sind die Exkretionsorgane nicht imstande,

die gebildeten N-haltigen Stoffwechselendprodukte in genügendem Masse nach aussen zu schaffen. Ausserdem muss man annehmen, dass die Süsswassertiere infolge der osmotischen Bedingungen ihres Aussenmediums rein passiv einen bestimmten Blutdruck und Turgor besitzen, die marine Organismen nur durch aktive Wasseraufnahme aufrechterhalten. Diese letztere Fähigkeit ist aber anscheinend zahlreichen Süsswassertieren verloren gegangen, und sie sind schon aus diesem Grunde nicht in Brack- und Meerwasser lebensfähig[1].

Eine andere biologische Erscheinung, zu deren Deutung zahlreiche Hypothesen aufgestellt worden sind, die aber erst im Zusammenhang mit der Erforschung der Osmoregulation der Wassertiere eine 'begründete Erklärung gefunden hat, ist das beim Vordringen mariner Tiere in das Süsswasser festgestellte Aufsuchen sauerstoffreicher Gebiete (Thienemann, 1928) und die Reduktion von Atmungsorganen bei Chironomiden- und Culicidenlarven sowie bei Hydracarinen mit zunehmendem Salzgehalt im Aussenmedium (Lenz, 1920, 1926, 1930; Martini, 1923; Thienemann, 1928). Erst die Feststellung, dass manche marine Evertebraten in Brack- bezw. Süsswasser im Zusammenhang mit dem Auftreten einer Osmoregulation einen erhöhten Sauerstoffverbrauch haben (Schlieper, 1929, 1930), brachte eine Lösung dieser Probleme.

Ein weiteres Problem ist die starke Einwanderung mariner Tiere in die tropischen Binnengewässer. Es ist hierbei besonders die Frage zu diskutieren, ob die höhere Wassertemperatur der Faktor ist, welcher die Osmoregulation in dem tropischen Süsswasser erleichtert. Ich möchte eine bejahende Antwort geben und annehmen, dass das Problem der tropischen Süsswassereinwanderung nur einen Spezialfall des allgemeinen in Kapitel VI erörterten Problems "Atmung und Osmoregulation" darstellt. In den gemässigten Breiten beobachten wir—wie oben erwähnt—bei manchen marinen Einwanderern in das Brack- und Süsswasser eine erhöhte Atmung. Da eine Verhinderung dieser Atmungssteigerung die Osmoregulation aufhebt bezw. erschwert, muss man schliessen, dass eine Osmoregulation bei Hypotonie des Aussenmediums von vielen Arten nur bei einer gewissen Mindestintensität des Gesamtstoffwechsels geleistet werden kann. Diese Mindestintensität des Stoffwechsels wird in unseren kühleren Breiten durch Erhöhung des Sauerstoffverbrauches erreicht, zu der allerdings nur ein kleiner Teil der marinen Tiere in der Lage ist. In den Tropen ist jedoch eine solche Erhöhung des Sauerstoffverbrauches bei Einwanderung in das Süsswasser nicht nötig, weil der Stoffwechsel der marinen Tiere dort schon infolge der hohen Wassertemperatur die für die Osmoregulation notwendige Mindestintensität hat.

VIII. ZUSAMMENFASSUNG.

Die osmoregulatorischen Organe halten das Verhältnis von Wasser und Salzen im Körper in einem dynamischen Gleichgewicht. Sie gewährleisten dadurch eine Konstanz des normalen kolloidalen Zustandes des Protoplasmas.

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[1] Damit hängt auch folgende Erscheinung zusammen: Führt man *Potamobius fluviatilis* aus Süsswasser in Meerwasser über, so stirbt er an "Austrocknung" infolge des osmotischen Wasserentzuges, weil er nicht die Fähigkeit hat, durch Trinken oder durch Aufnahme von Seiten der Haut die verlorenen Flüssigkeitsmengen zu ersetzen.

Osmoregulatorische Fähigkeiten besitzen alle Süsswasserbewohner, zahlreiche Brackwassertiere, die marinen Elasmobranchier und die marinen Teleostier. Alle Süsswasserevertebraten und die stenohalinen Süsswasserfische sind in Meerwasser nicht imstande, die Eigenkonzentration ihres Innenmediums zu bewahren; das gleiche gilt für die stenohalinen Meeresbewohner nach Überführung in Süsswasser. Dagegen sind die euryhalinen Meeresevertebraten in Brackwasser und zum Teil auch in Süsswasser homoiosmotisch. Die euryhalinen Teleostier sind in Meer- und Süsswasser homoiosmotisch.

Die Exkretionsorgane zahlreicher Süsswassertiere funktionieren osmoregulatorisch, indem sie durch Ausscheidung eines verdünnten salzarmen Harnes den osmotischen Wassereinstrom durch die Körperoberflächen kompensieren. Der Bau der Exkretionsorgane lässt sich bei zahlreichen Evertebraten und Teleostiern in gleicher Weise in Beziehung zu ihren osmoregulatorischen Leistungen bringen. Während bei den Exkretionsorganen der Süsswassertiere häufig die Filtrations- und Rückresorptionsabschnitte relativ stark entwickelt sind, überwiegen bei den marinen Tieren ebenso häufig die sekretorischen Abschnitte. Bei einigen niederen Wassertieren (Turbellarien und Arthropoden) lässt sich eine osmoregulatorische (wasserhinausschaffende) Funktion des Darmkanales nachweisen.

Die Permeabilität der Körperoberflächen der wasserlebenden Tiere steht in Beziehung zu den osmoregulatorischen Leistungen.

Eine neue Arbeitshypothese besagt, dass die Kiemen bezw. die Körperoberflächen homoiosmotischer Brackwasserevertebraten Salze entgegen dem osmotischen Gefälle von aussen nach innen transportieren und so den Exkretionsorganen die Bildung eines blutisotonischen Harnes ermöglichen.

Die marinen Teleostier trinken regelmässig Seewasser, während ihre Kiemen die überschüssigen Salze in Form einer konzentrierten Lösung nach aussen transportieren, und ihre Nieren geringe Mengen eines annähernd blutisotonischen Harnes ausscheiden. Auch bei den marinen Elasmobranchiern ist eine extrarenale Ausscheidung von Salzen wahrscheinlich gemacht worden.

Exakte Messungen des osmoregulatorischen Energieverbrauches eines Wassertieres existieren noch nicht. Es sind aber Beziehungen zwischen dem Salzgehalt des Aussenmediums und dem Sauerstoffverbrauch bei zahlreichen Wassertieren gefunden worden, die wahrscheinlich mit der Osmoregulation und dem Hydratationsgrad der Gewebe im Zusammenhang stehen.

Das Studium der osmoregulatorischen Eigenschaften der Wassertiere hat wesentlich zum Verständnis ihrer Biologie und Ökologie beigetragen. Die Besiedlung des Brack- und Süsswassers von Meere her ist nur durch die Ausbildung osmoregulatorischer Mechanismen möglich gewesen. Die meisten Süsswassertiere sind selbst in isotonischem Brackwasser auf die Dauer nicht lebensfähig, weil ihre Gewebe und ihre Exkretionsorgane auf einen osmotischen Wassereinstrom durch die Haut eingestellt sind. Fehlt bei den Süsswassertieren dieser Wassereinstrom in isotonischem Brackwasser oder ist er in hypotonischem Brackwasser vermindert, so sinkt der Turgor ihrer Gewebe, und ihre Exkretionsorgane können die N-haltigen Stoffwechselendprodukte nicht mehr ausscheiden.

IX. SUMMARY.

Osmoregulatory organs maintain the proportion of water to salts in the body in a state of dynamic equilibrium. They thus assure the constancy of the normal colloidal state of protoplasm.

All fresh-water and numerous brackish-water animals, the elasmobranchs, and the marine teleosts, have osmoregulatory powers. No fresh-water invertebrates or stenohaline fresh-water fishes are able to maintain the normal concentration of their internal medium in sea water, and the same is true of stenohaline marine animals when they are put into fresh water. On the other hand, euryhaline marine invertebrates are homoiosmotic in brackish water, and some of them in fresh water. Euryhaline teleosts are homoiosmotic in sea water and in fresh water.

The excretory organs of numerous fresh-water animals act as osmoregulators in that they compensate the osmotic intake of water through the surface of the body by the excretion of a dilute urine poor in salts. In numerous invertebrates and teleosts the structure of the excretory organs corresponds with their osmoregulatory functions. Whereas in the excretory organs of fresh-water animals the filtering and resorbing portions are often relatively strongly developed, in marine animals the secretory part is equally often the most prominent.

The permeability of the body surfaces of aquatic animals is correlated with their osmoregulatory powers.

The gills or body surfaces of homoiosmotic brackish-water invertebrates transport salts against the osmotic gradient from outside to inside the body, thus allowing the excretory organs to form urine isotonic with the blood.

Marine teleosts drink sea water, while their gills transport the excess salts outwards in the form of a concentrated solution, and their kidneys excrete small amounts of almost isotonic urine. It is probable that extrarenal excretion of salts occurs also in marine elasmobranchs.

Exact data are not yet available of the osmoregulatory energy requirements for any aquatic animal. But in numerous cases relationships have been found between salt content of the external medium and oxygen consumption, which are probably connected with osmoregulation and the state of hydration of the tissues.

The study of the osmoregulatory characteristics of aquatic animals has contributed to the knowledge of their biology and ecology. The colonisation of brackish and fresh waters from the sea has only been possible thanks to the development of osmoregulatory mechanisms. The majority of fresh-water animals cannot live even in isotonic brackish-water, because their tissues and their excretory organs require an osmotic stream of water. If the latter fails in isotonic brackish water, or if it is diminished in hypotonic brackish water, the turgor of the tissues falls and the excretory organs can no longer eliminate nitrogenous waste.

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[ANM. Die Arbeiten von Schlieper, Herrmann, Nagel, Peters, Pieh, Scholles und Schwabe sind Glieder einer zusammenhängenden Untersuchungsreihe, die im Zoologischen Institut der Universität Marburg (Lahn) entstanden ist.]

THE COMPARATIVE PHYSIOLOGY OF COLOUR RESPONSE IN REPTILES AND FISHES

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CONTENTS.

I. INTRODUCTION.

THE phenomenon of colour change in animals has been known since ancient times, but our knowledge of the physiology of the process began only about a hundred years ago when Milne-Edwards discovered and described the melanophores in the skin of the chameleon. Before that the most fantastic theories had been advanced, among which one may cite Cuvier who stated that the chameleon changes colour by distending his lungs with air. Our conception of the melanophore as a contractile cell has been modified in only one important respect since the work of Milne-Edwards. He described it as a vesicle filled with granular pigment, and capable of expansion and contraction rather in the manner of an amoeba. The extensive histological researches of Ballowitz (1893), and in particular the recent microdissection studies of Matthews (1931), have shown that this picture is incorrect, and that in fact the pigment cell has a permanent arborising contour within which the granular pigment migrates by a process of aggregation and dispersion. Apart from this the microphysiology of the pigmentary effector unit remains entirely obscure.

The ability to change colour is shared by most cold-blooded vertebrates, some crustaceans, some leeches, and the cephalopod molluscs. Among the vertebrates

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the structure and arrangement of the pigment cells are fairly uniform, though differences in detail exist. In addition to melanophores these animals possess chromatophores containing red and yellow pigment which is soluble in alcohol, acetone and other lipoid solvents, and also iridescent cells containing guanin crystals. These are the units which compose the brightly coloured patterns and markings which these animals display. Whether they are always all contractile is by no means sure, though certainly in fishes there is ample evidence that the xanthophores and erythrophores contribute to the changes in colour and marking of the skin that may occur. But the melanophores are undoubtedly the most significant and the most universal elements in colour response. By their expansion they not only serve to darken the skin, but they also obliterate the bright pigmentation due to the other chromatic elements, thus providing for a very wide range of colour changes.

The physiological study of colour change suffers from one ineluctable difficulty. The pigmentary effector system is comparable to the muscular apparatus in complexity, but, whereas the muscles can be accurately specified anatomically, and their tension, that is their degree of physiological activity, can be kymographically recorded and measured with precision, the chromatophores do not lend themselves readily either to anatomical classification or to physiological measurement. The only method at present available of recording their activity is by visual estimation of their degree of expansion and contraction. This method is questionable enough when the whole skin displays a uniform shade, but when, as more frequently happens, there is a complicated and many coloured pattern, it is particularly inadequate. Hewer (1926) has emphasised the striking lack of co-ordination in the reactions of the chromatophores of the dab, and has pointed out that it implies a measure of independence in the activity of different regions of the skin. It follows, therefore, that when we say that certain environmental conditions produce melanophore contraction, or expansion of the xanthophores, such statements are no more than rough approximations to a statistical average with a very high standard deviation. On account of the fact that the experimental results can be expressed only in terms of visual quantitative estimates—which may be called "more-or-less" recording—the investigator of pigmentary activity is peculiarly susceptible to auto-suggestion, and such statements as that "the operated, or injected animals were slightly darker than the controls" should be accepted with caution. In view of these considerations it is not surprising to find that the literature of colour response contains many contradictions.

Following on the discovery of Milne-Edwards, Brücke in 1852 carried out his classical experiments on the chameleon, and showed that the melanophores of this animal are controlled by nerves. Pouchet, twenty years later, in a magnificent monograph which was awarded the Prix de physiologie expérimentale of the Académie des Sciences, demonstrated the role of the nervous system in the colour changes of fishes, and von Frisch (1911 c, 1912) carried on the analysis of nervous function. These are the landmarks. About twenty years ago the question of the possible endocrine control of colour change began to be raised, and was definitely answered, so far as the Amphibia are concerned, by the work of Hogben and

Winton (1922), who showed that pigmentary activity in this group is controlled by the internal secretions of the pituitary gland. This discovery, which has been strengthened and extended by the subsequent work of Hogben and his associates on *Xenopus laevis*, stimulated intensive endocrinological researches in the other colour-changing groups. So far they have been entirely successful only in the case of Crustacea, where, as in Amphibia, the nervous system is now known to be concerned in colour change only to a very small extent. Among reptiles and fishes the exact function of endocrine agencies is still uncertain. The work of Redfield (1918) on the American lizard (*Phrynosoma*) claimed to establish the dominant function of the adrenal organs in the colour change of this animal, and his work has greatly influenced the literature of comparative pigmentary physiology during the last ten or fifteen years, but the results of the investigations of Hogben and Mirvish (1928) and of Zoond and Eyre (1934) have failed to confirm Redfield's conclusions, and consequently the case for the role of the adrenals in reptilian colour responses is now very weak indeed. In fishes, on the other hand, although the controlling activity of the nervous system has never been called in question, much evidence is accumulating that there is also a certain measure of endocrine control.

The nineteenth-century investigators proceeded on the assumption that the mechanism of colour change was fundamentally uniform in all the colour-changing groups. The work of the present century has shown this assumption to be false, and has emphasised the profound divergence in the pigmentary physiology of different animals. Few recent authors have attempted to treat the subject from a broadly comparative point of view. Parker's article in *Biological Reviews* (1930), as well as his more recent book (1932), summarises the existing literature of the subject, but more recent work has changed and extended our conceptions in several respects, and has furnished the materials for the present article, which is an attempt to compare the processes of pigmentary response in fishes and reptiles with the aim of formulating a comprehensive statement of the physiological processes by which the pigmentary activity of these two groups is co-ordinated.

II. THE MELANOPHORES.

To divide a physiological discussion on the basis of anatomical units is perhaps an unusual and arbitrary procedure. It is clear that all the chromatophores together are concerned in any particular kind of chromatic response. Yet there are many reasons why it is convenient to treat the melanophores separately from the coloured pigment cells in a comparative study such as this. The melanophores are, in general, the dominant elements in colour change; the principal variations in the shade and colour of the skin are due to melanophore expansion and contraction more than to the activity of other cells. Secondly, the physiology of the melanophores is much better known; in fact in the reptiles the function of other elements has scarcely yet been analysed. And, lastly, it is with respect to melanophore activity that the most fruitful comparison between the two classes can be drawn.

<center>(1) Nervous control of melanophore activity.</center>

The innervation of the melanophores has been histologically demonstrated in fishes by Ballowitz (1893), and in lizards and snakes by Leydig (1873). Physiologically, it was proved by Pouchet (1876) that section of nerves in the turbot results in darkening of the denervated skin region. Von Frisch (1911 c) confirmed this and proved that the melanophores are supplied by fibres belonging to the autonomic system. On the basis of exhaustive experiments he worked out the anatomical re-
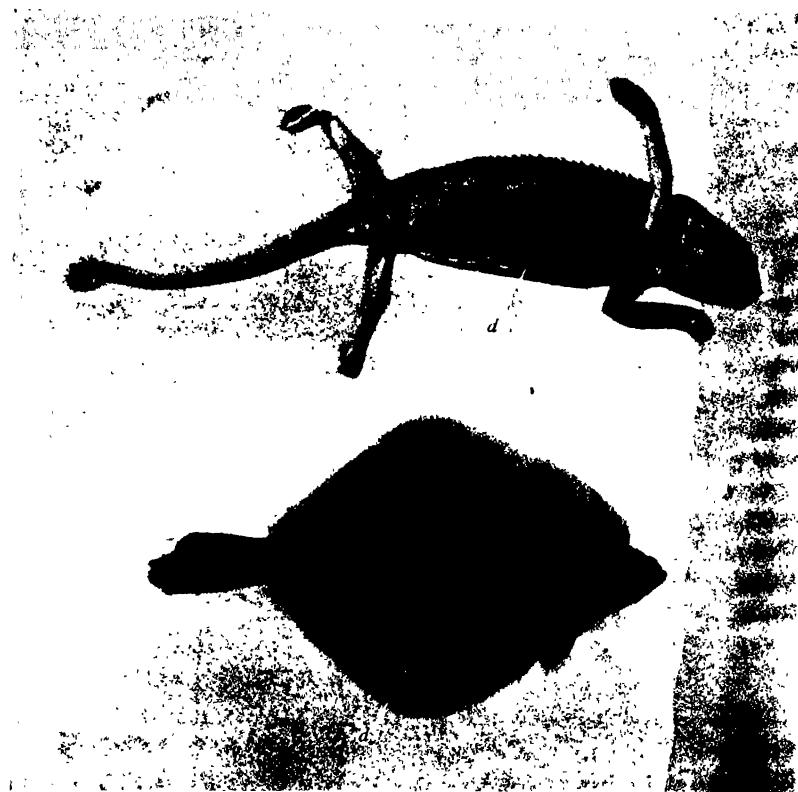
Fig. 1. The effect of spinal nerve section in the plaice and the chameleon. The melanophores in the denervated area (d) are expanded, in the rest of the skin they are mostly contracted.

lations of the nerve paths that link the melanophores with the central nervous system. His results have received repeated confirmation with reference to many different species of fishes (Wyman, 1924; Hewer, 1927; Giersberg, 1930; Fries, 1931; Smith, 1931). In reptiles, Brücke (1852) was the first to show that the melanophores of the chameleon are under the control of the autonomic nervous system. Here also nerve section results in melanophore expansion in the denervated region. Fig. 1 shows the effect of spinal nerve section in the chameleon

and the plaice. Brücke's results have been confirmed by Bert (1875), Keller (1895), Hogben and Mirvish (1928), and Zoond and Eyre (1934), all working on different species of chameleons. For other reptiles little evidence on this point has been advanced. Redfield's data (1918) for the American lizard (*Phrynosoma*) are contradictory. Section of nerves was without effect, but electrical stimulation of the central nervous system after adrenalectomy resulted in melanophore contraction. Carlton (1903) found that the melanophores in the excised skin of the lizard (*Anolis carolinensis*) are contracted, and Hadley (1928) showed that in *A. equestris* the excised skin is dark, but becomes pale when removed from the light. These are puzzling results which it is impossible to reconcile with the data obtained from chameleons.
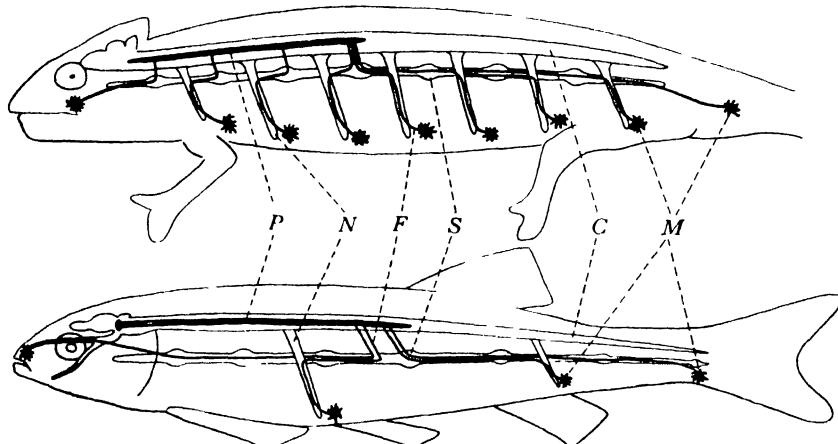
Fig. 2. Diagram showing the nervous control of melanophores in the minnow (below), and the chameleon (above) (the minnow after von Frisch). *C*, spinal cord; *F*, pigmentomotor fibre; *M*, melanophore; *N*, spinal nerve; *P*, pigmentomotor centre; *S*, sympathetic.

Hogben and Mirvish (1928) have analysed the connection of the melanophores with the central and peripheral nervous system in the chameleon. Fig. 2 presents, for comparison, von Frisch's diagram of nerve paths in the minnow (*Phoxinus*), and a similar diagram for the chameleon, based on Hogben and Mirvish's results. The similarity is striking, the chief difference being that in the fish the preganglionic fibres leave the spinal cord only at the 15th vertebra, whereas in the reptile they emerge segmentally down to the 12th or 13th.

Electrical stimulation of nerves in fishes has always been found to cause the melanophores to contract. The same has been reported for the chameleon by all those who have worked on this animal. In *Phrynosoma*, Redfield could produce melanophore contraction in the leg by stimulation of the sciatic, but stimulation of spinal nerves gave no result. So far as I am aware no tests of this kind have been carried out on other reptiles. It seems certain, therefore, that, so far as fishes and chameleons are concerned, the discharge of efferent nerve impulses causes the melanophores to contract.

Von Frisch (1911 c) found that in *Phoxinus* pallor could be produced by stimulation of the anterior portion of the spinal cord or of the medulla. Stimulation of the midbrain, however, or of the optic lobes had no effect. Stimulation of the forebrain was found to cause melanophore expansion, from which it was concluded that an inhibition centre existed in this region of the brain. These experiments were repeated by Schaefer (1921) on the plaice (*Pleuronectes*). He failed to produce darkening by stimulation of any region of the brain, but in all other respects he confirmed von Frisch's results. Further confirmation was supplied by Spaeth (1916) and Wyman (1924) for *Fundulus*. In reptiles—the chameleons and *Phrynosoma*—it was shown by Redfield and by Hogben and Mirvish that tetanisation of the roof of the mouth causes generalised pallor. Curiously enough they have named this effect "excitement pallor," an unsuitable term, because the electrical stimulation of the roof of the mouth undoubtedly causes direct central nervous excitation, and does not differ qualitatively from the stimulation of any other part of the nervous system. A response to such stimulation is no more an "excitement" effect than the tetanic contraction of the somatic muscles following central nervous stimulation. It will be shown later that, in so far as it is possible to define emotional states in reptiles at all, excitement appears to manifest itself in darkening of the skin rather than pallor. In these reptiles, also, the stimulation of the spinal cord in the anterior part of the body causes melanophore contraction. It seems most probable, therefore, that the stimulus applied to the roof of the mouth exerts its influence on the medulla, as it does in fishes, but no direct tests with brain stimulation similar to von Frisch's experiments have been recorded.

In recent years several authors have revived the idea first suggested by Bert (1875) that the melanophores receive a double innervation of sympathetic and parasympathetic fibres, which control their contraction and expansion respectively. Bert, it is true, applied this idea to chameleons, and published no experiments. In its recently revived form the theory has been applied to fishes. Giersberg (1930) supports his argument by experiments with drugs. Injection of parasympathomimetic substances like pilocarpin caused melanophore expansion in the minnow. The same effect resulted from electrical stimulation of the medulla or of the sympathetic chain following administration of ergotamine and acetylcholine.

The chief protagonist of the double innervation theory is Parker (1934 a, b). His theory rests on an entirely novel interpretation of the physiological results of nerve cutting. Pouchet (1876) and von Frisch (1911 c) spoke of the region of the skin whose nerve supply had been severed as the *paralysed* region, and they regarded the melanophores of such a region as being unaffected by pigmentomotor discharges issuing from the central nervous system. They were therefore unexcited melanophores. Such a view has been accepted by almost all the authors cited in this review. Parker and Porter (1933), however, take a totally different view. They state that the darkening of a denervated skin region in *Fundulus* "is with reason believed to be due to the mechanical stimulation of cutting which is more effective for the expanding nerve fibres than for the contracting ones." This statement is based on the work of Mills (1932 a) from Parker's laboratory. She found that mechanical stimulation of

the melanophore nerves in *Fundulus* causes melanophore expansion. These authors appear to be entirely satisfied that section of an efferent nerve will set up a prolonged discharge of impulses in the peripheral stump, and in a later paper (Parker and Porter, 1934) they apply the term "exciting incision" to such an operation. In this paper, which deals with the dogfish (see p. 381), the conception of prolonged stimulation of pigmentomotor nerves as a result of cutting is extended to contracting, that is, sympathetic fibres. The following quotation summarises this point of view: "in our opinion the fin nerves concerned with melanophore contraction in the dogfish must be regarded as in more or less continuous action as a result of the irritation of the wound for a period of at least five days" (Parker and Porter, 1934).

Although such an opinion is contrary to traditional physiological principles, Parker (1934 *c*) is convinced that it is justified by the evidence which he is able to advance in support of it. The evidence is that when, in a denervated tail region which after several days has become pale (see p. 376), "the nearly faded band is recut in a region distal to the first cut, its activity is fully demonstrated by a quick revival of considerable depth of colour. Such observations show quite conclusively that the radial nerves of the tail fin in these fishes... carry nerve fibres whose normal action on their associated melanophores is to produce a dispersion of pigment" (Parker, 1934 *a*). In an earlier paper, however, it appears that a similar experiment was performed with the opposite result (Parker and Porter, 1933). Here a second transverse cut was made in the tail posterior to the first cut, and five days later, when the longitudinal denervated dark band was still slightly visible, the authors state that "the fact that the melanophores are no more expanded in the region posterior to the secondary cut than they are in that anterior to it shows that the whole band is at this stage still denervated and has not yet been invaded, at least in the region of the secondary cut, by regenerating nerve fibres."

This question would be settled once and for all if it were possible to demonstrate a continuous discharge of action potentials in the peripheral portion of a transected motor (or fixed) nerve. Parker (1934 *c*) quotes Adrian (1930) to the effect that a post-operative discharge of impulses occurs in mammalian medullated nerve, and he also refers to the observation of Hoagland (1933) that there is a spontaneous discharge of afferent impulses in the lateral line nerves of fishes. With regard to Adrian's work, it appears to me that his observations offer little support to Parker's thesis, because, although he found that in excised mammalian nerve discharges of varying frequency and rhythm may last for an hour or more, such discharges appeared to be confined to sensory fibres, and, moreover, no activity could be demonstrated in the excised nerves of cold-blooded vertebrates, except during the actual infliction of an injury. The spontaneous activity of the lateral line nerve is again a phenomenon which bears little relation to the supposed discharge of impulses in the peripheral portion of a transected nerve trunk. The impulses in this case originate in the sense organs of the lateral line, and I have convinced myself that the spontaneous discharge (assuming that it *is* spontaneous, and is not due to some stimulus hitherto unidentified) ceases when the connections of the nerve with its end-organs are severed. I have found, moreover, that the *excised* lateral line nerve is completely

inactive, and that a fresh cut initiates an outburst of impulses which subsides in rather less than a minute. The activity of the lateral line may be compared, perhaps, with the behaviour of one of the spontaneous "centres" in the central nervous system, but it certainly provides no analogy to the case of a transected nerve.

It must be admitted, therefore, that the argument for the double innervation of fish melanophores is open to serious criticism. Yet it is this argument which forms an integral part of the theoretical basis of Parker's hypothesis of neurohumours. His evidence for a cell-to-cell transmission of substances secreted by nerve endings is derived mainly from a consideration of what happens to the melanophores in a denervated tail region of a fish. The following experiment, for example, may be considered: "if an almost fully faded caudal band is flanked on each side and for about half its length by newly excited dark bands, the part of the faded band proximal to the flanking dark bands remains indefinitely light whereas that between the two bands gradually darkens.... It is difficult to explain this darkening of the distal portion of the faded band except on the assumption that a dispersing neurohumour has made its way from the flanking dark bands into the faded one" (Parker, 1934 a).

We may agree that it is difficult to explain this phenomenon on any other assumption, but we must bear in mind that this assumption requires a series of antecedent assumptions, namely, that

(1) the melanophores receive a double innervation of contracting and expanding fibres (Mills, 1932 a; Parker, 1934 a),

(2) cutting the nerves sets up a sustained injury discharge in the expanding, but not in the contracting, fibres (Parker, 1934 b), and

(3) electrical stimulation of peripheral nerves excites the contracting, but not the expanding, fibres (Parker, 1934 b).

(2) The question of independent effector activity.

It is now necessary to enquire to what extent melanophore activity can be caused by agencies other than autonomic nervous impulses and the neurohumours which they possibly liberate at the peripheral nerve endings. The list of possible agencies is extensive and heterogeneous. Temperature, pressure, light, salts, drugs, narcotics, oxygen tension, hydrogen-ion concentration and hormones may all be considered in this connection. But it is not necessary to deal with them all in a discussion of the independent effector activity of melanophores. An independent effector may be defined as an organ which combines the functions of a receptor and an effector in that it is able to respond to external stimulation without the intervention of a reflex mechanism. In so far as concerns our present purpose it will be sufficient to consider the relation of melanophores to light and temperature.

The question of the direct activation of melanophores by light was first raised in connection with the well-known phenomenon in chameleons of the local darkening of illuminated skin. The simplest way to explain this response appeared to be that the melanophores were acting as independent effectors, and this view was adopted by Bert (1875), Keller (1895), and Redfield (1918). Zoond and Eyre

(1934) showed, however, that when a decapitated chameleon is opened by means of a dorso-lateral incision, and the heart and viscera are removed, the preparation will continue to respond to light only on the intact, not on the transected, side. Since, in this experiment, the possibility of the intervention of uncontrolled agencies, such as interference with the circulation, was automatically excluded, they concluded that the response of the melanophores to skin illumination is a spinal reflex response. On the other hand, it appears that there is at least one reptile whose melanophores react to light even when their nervous connections are severed. Hadley (1928) discovered that the excised skin of the lizard (*Anolis equestris*) responds to illumination by melanophore expansion. It is difficult to see how there could be any possibility of error in a direct positive observation of this kind, and yet, as Zoond and Eyre have pointed out, the additional observation supplied by Smith (1929) that the same skin responds also to heat by melanophore *contraction* renders Hadley's observation unintelligible. Zoond and Eyre maintain that "on purely theoretical grounds, an independent effector which is found to respond to photic and thermal stimulation should respond to both kinds of stimuli in the same way." Their argument is that a contractile structure must display a resting and an active phase, and any agency that supplies an adequate stimulus to it must necessarily evoke the active phase. Parker (1930) was inclined to accept the evidence of Redfield and Hadley for the direct response of reptilian melanophores to light.

In fishes, a local response to illumination of the skin has been reported only in one species. Von Frisch (1912) found that when a spot of light was focused on the skin of the wrasse (*Crenilabrus*), a sharply defined dark area appeared after a few seconds. He could elicit no such reaction, however, in the gurnard (*Trigla*) or the minnow (*Phoxinus*). Nor could Schaefer demonstrate it in the plaice, or Mast (1914) in the flounders (*Paralichthys* and *Ancylopsetta*). Spaeth (1913) observed that isolated scales of *Fundulus* immersed in 0·1 M NaCl, the melanophores being expanded, were unaffected by visible light, but when exposed to ultra-violet radiation of wave-length 185 290$\mu\mu$, the melanophores contracted rapidly and reversibly.

Thus, so far as the evidence goes, it appears that both among fishes and reptiles there are some species whose melanophores are unaffected by direct illumination when deprived of their nervous connections, while in others there is an indication that such power of direct reactivity exists. Undoubtedly, further investigation of this fundamental point is urgently needed.

Still less evidence exists concerning the effect of heat, either directly on the denervated melanophore or reflexly on the intact animal. In *Phoxinus* von Frisch (1911 a) found that the application of warmth to one side of the intact animal caused a darkening of that side, but in the decapitate and pithed preparation heat caused melanophore contraction. Similar results were reported for *Fundulus* by Smith (1928). The reaction of the pithed preparation was interpreted by von Frisch as "Anämieaufhellung," but Spaeth (1913) maintained that heat is a specific contracting agent for *Fundulus* melanophores, independent of lowered oxygen tension.

The effect of temperature on reptilian melanophores is discussed by Zoond and

Eyre. There is agreement among all authors who have dealt with this subject that exposure to warmth causes pallor in reptiles. The melanophores in excised skin react in the same way, but apart from the observations of Smith (1929) on *Anolis equestris*, there is no evidence that this response is reversible, and differs qualitatively from the heat rigor that must develop in any contractile structure at sufficiently high temperatures. It would be rash, therefore, to assert, on the data at present available, that reptilian melanophores act as independent effectors with respect to heat.

The question of the influence of low temperature raises difficult theoretical issues, and it cannot be said that any agreement on this matter has been reached hitherto[1].

(3) *Response to background.*

Many species of fish possess, to a varying extent, the power to adapt to the shade, colour and pattern of the background. According to Parker (1932) this fact was first recorded in biological literature in 1830 by Stark, though, as Pouchet remarked, it must have been known to fishermen long before that. The adaptation to colour, which, of course, is due to the activity of the coloured chromatophores, takes place much more slowly than adaptation to shade. It will be dealt with in Section III. The power to reproduce the pattern of the background occurs to a marked extent in flounders only. Excellent photographs of this have been published by Sumner (1911) and Mast (1914). The former worked with *Rhomboidichthys*, the latter with *Paralichthys* and *Ancylopsetta*. The pattern responses of these fishes are striking evidence of the complexity of melanophore control, since they undoubtedly depend upon a differential contraction of the melanophores in different regions of the skin. The nature of this control is entirely unknown. Mast is of the opinion that the relative areas of light and dark regions in the background determine the chromatic response, and that their form and arrangement have little effect.

A very curious fact which first attracted the attention of Pouchet, and upon which many subsequent investigators have commented, is that "practice" reduces the time factor in the response of a number of fishes to dark- and light-coloured backgrounds. The same applies, possibly, also to colour adaptation. Thus Sumner records that one of his *Rhomboidichthys*, after several changes of background, "learnt" to adapt within a fraction of a minute, whereas at first it had required some hours. At the same time it must be noted that different species of fish vary enormously in the rapidity with which they can adapt to the shade of the background. Meyer (1931) found that the spotted goby (*Gobius ruthensparri*) adapts in less than a minute, while the little goby (*G. minutus*), like the *Pleuronectides*, requires several hours.

Background adaptation in reptiles was unknown until 1918 when Redfield observed it in the lizard (*Phrynosoma coruntum*). Zoond and Eyre have analysed the response to white and black background in the chameleon. In this animal adaptation to black occurs in 3 or 4 min., but adaptation to white requires a somewhat

[1] A discussion of this matter appears on pp. 38–41 of Zoond and Eyre (1934).

longer time. It was found also that the background response is conditioned by the intensity of illumination, inasmuch as weak (artificial) light induces a *darkening* on a white as well as on a black background, a response which, they were able to show, is dependent, like other background responses, upon visual stimuli. This result is the more surprising since in fishes the background responses have always been found to be independent of intensity of light. Zoond and Eyre did not carry out exact experiments with photometrically controlled intensities, and until this has been done the significance of the phenomenon they observed must remain obscure. Nevertheless, their work has disclosed the fact that reptiles are no exception to the general rule that adaptation to the shade of the background is a dominant feature of the pigmentary activity of colour-changing animals.

(4) *Response to darkness.*

Those workers who have investigated colour change in fishes have been pre-occupied with the responses to different kinds of background, while those who have worked on reptiles have been concerned mainly with the effect of direct illumination of the skin. Thus it has come about that to-day we have fairly extensive data on the state of reptilian melanophores when the animal is in darkness, while our knowledge on the same point with respect to fishes is meagre. And yet this is a matter concerning which it is essential to have precise and unequivocal information before we can proceed with the formulation of a general theory of pigmentary co-ordination. For it is only in total darkness that the pigmentomotor apparatus is known to be in a totally unstimulated condition, and we can only discuss co-ordination fruitfully when we know all the individual effective stimuli operating on the animal in any given environmental situation.

The evidence for different species of *Chameleo, Anolis* and *Phrynosoma* all goes to show that the melanophores of reptiles are contracted when the animals are equilibrated to darkness (Brücke, 1852; Keller, 1895; Carlton, 1903; Parker and Starratt, 1904; Redfield, 1918; von Geldern, 1921; Hadley, 1928; Zoond and Eyre, 1934). With regard to fishes, unfortunately, there is less agreement. The earliest observation appears to be that of Mayerhofer (1909) who found that the pike (*Esox lucius*) becomes pale in the dark, and regains its "normal colour" when returned to the light. Von Frisch also has definitely stated in several places that the minnow (*Phoxinus laevis*) (1911 c) when put in the dark goes pale after a few minutes, and he makes the same statement concerning the wrasse (*Crenilabrus*) (1912). Finally, Parker and Lanchester (1922) record that *Fundulus* is pale in the dark. These fishes, therefore, exhibit the same behaviour as reptiles. On the other hand, a number of authors have recorded that other fishes retain a more or less dark shade when removed from the light. These are listed in Table I.

(5) *Effect of blinding.*

The background responses of fishes and reptiles are, of course, abolished when the eyes are excised or the optic nerves are cut. Blind chameleons and lizards, however, still retain in part their chromatic function by virtue of their ability to respond

to direct illumination of the skin. Their melanophores are contracted in the dark, and expand when exposed to the light. The same is true of those fishes that have been found to show pallor in darkness, and there is the additional observation of Secerov (1909) that *Nemachilus barbatula*, when blinded, is paler in the dark than in the light. Some other fishes, however, are found to darken after blinding, and to remain in this condition whether exposed to the light or not (see Table I).

From the foregoing we may conclude that although all fishes do not show the same behaviour after blinding, it is possible to equate some fishes with the reptiles in this respect. This raises the important question of the nature of the mechanism which determines the chromatic response of blind animals to light. In chameleons it has been shown by Zoond and Eyre that the phenomenon is a spinal reflex involving the activity of dermal photoreceptors. In *Phoxinus* von Frisch (1911 *c*)

Table I. *Condition of normal and blind fishes in the dark.*

Normal		Blind	
Pale	Dark	Pale	Dark
Fundulus (Parker, 1922) *Phoxinus* (von Frisch, 1911) *Cremlabrus* (von Frisch, 1912) *Esox* (Mayerhofer, 1909)	*Amiurus* (Bray, 1918) Flounder (Cunningham, 1893) Several sp. (Kudo, 1922)	*Fundulus* (Parker, 1922) *Phoxinus* (von Frisch, 1911) Cyprinoids (von Frisch, 1912) *Nemachilus* (Secerov, 1909)	*Amiurus* (Bray, 1918) Several sp. (Kudo, 1922)

found that the response was not a local one to light falling upon the skin, but that it depended upon the illumination of the top of the head. He was able to show that the response persisted after the whole head integument had been removed; and even when the pineal organ was cut out, a pencil of light focused upon the top of the brain induced general expansion of melanophores all over the body. From this he drew the conclusion that this species possesses a functional parietal organ, though the exact locus of the photosensitive cells remained somewhat in doubt. In *Crenilabrus*, however, the reaction is local as in chameleons (von Frisch, 1912), though it was not determined whether the response in this animal was a direct or a reflex one.

Although these observations are not as broadly comparative as could be wished, they furnish clear evidence that both among reptiles and fishes there exist, in addition to the eyes, photoreceptive elements whose stimulation by light causes melanophore expansion in blinded animals.

(6) *Co-ordination of pigmentary responses to light.*

When *Phoxinus* is compared with the chameleon it is found that they have the following properties in common:

(1) Nerve stimulation causes melanophore contraction. The innervation is autonomic.

(2) Nerve section causes expansion in the denervated region.

(3) Both show adaptation to the shade of the background, but lose this power when deprived of vision.

(4) Both become pale in the dark.

(5) Blind animals are pale in the dark and darken when exposed to light.

The parallelism is impressive. It must be borne in mind, however, that it can by no means be extended to include all fishes and reptiles, in the present state of knowledge. In so far as it is possible to formulate a theory of the co-ordination of nervous control of melanophore activity, it is obvious that the same reasoning will apply to both *Phoxinus* and the chameleon. When the animal is in darkness where reflex photic stimulation is absent, the melanophores appear to be maintained in a state of contraction (active phase) by a discharge of autonomic impulses from a centre in the central nervous system. When the animal is illuminated this discharge is reflexly inhibited, since the melanophores then expand (resting phase). In the chameleon, according to the findings of Zoond and Eyre, this inhibition is due to the stimulation of dermal photoreceptors, whereas in *Phoxinus*, according to von Frisch, it is due to a functional parietal organ. Incidentally it may be mentioned that von Frisch was able to locate the "Hemmungszentrum" in the brain by unipolar stimulation (1911 *b*). In the chameleon it has not been found possible to induce melanophore *expansion* by stimulation of any part of the central nervous system.

This inhibition of the autonomic discharge when the animal is illuminated must not be lost sight of when the co-ordination of background adaptation is discussed. If it exists at all, the afferent impulses from the dermal photoreceptors in the chameleon, or from the parietal organ in *Phoxinus*, must flow towards the centre whenever the animals are in the light, and whatever the nature of the background on which they happen to rest. The afferent discharge from the eyes is therefore superimposed upon or added to the discharge from extra-ocular photoreceptive elements.

It would appear that the inhibiting influence of this afferent discharge upon the autonomic efferent impulses is suspended when the animals show melanophore *contraction* in the light, *i.e.* when they are on a background of light shade. Zoond and Eyre have suggested that under such conditions the function of the afferent discharge from the retina is to inhibit the discharge from the dermal photoreceptors (in chameleons), which is itself an inhibiting discharge. The concept of an inhibition of inhibition is theoretically cumbersome, and it will not be easy to furnish direct evidence that such a process actually plays a part in central nervous activity. But when this interpretation of the white background response is adopted, it presents the possibility of experimental verification to a certain extent. For it implies that the white background response depends not only upon the visual stimulation received from the illuminated white background, but also upon extra-ocular stimuli due to light which falls directly upon the skin (the whole skin in chameleons, the top of the head in *Phoxinus*). If this is so, then it should be possible to eliminate the second source of stimulation while preserving the first. This has been done with chameleons (Zoond and Bokenham, 1935) by enclosing the body in a light-proof jacket and exposing the animals on a white background. The interpretation of the results is somewhat dubious, however, owing to the fact that the contracted condition of the melano-

phores is characteristic both of the state of adaptation to white background and of equilibration in darkness. Nevertheless there is a means of distinguishing the pallor which is a true white background adaptation from the primary pallor of the photically unstimulated animal in darkness. The latter, in chameleons, is much more intense, and presents an appearance of the skin which is never observed in animals in the light. Moreover, it is found that the transition from darkness pallor to white-adapted pallor involves a transient expansion of the melanophores. This has been interpreted as the manifestation of the primary inhibition before the secondary inhibition comes into effect, but it may be objected that the time relations are too
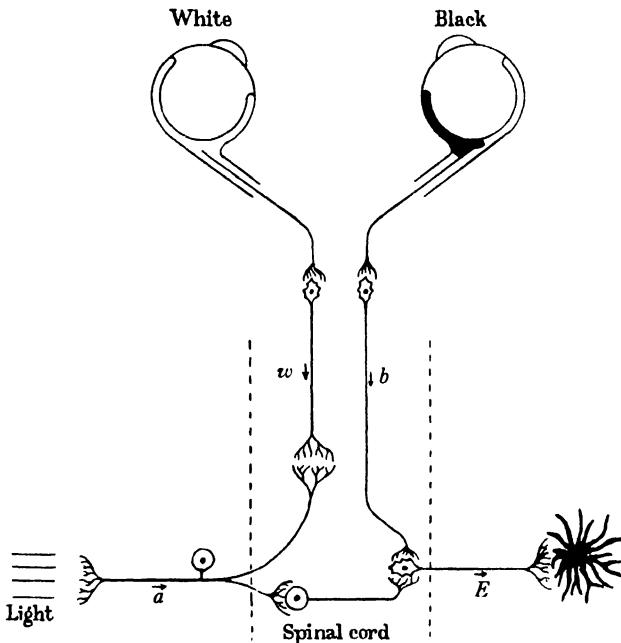
Fig. 3. Hypothetical scheme for the nervous co-ordination of melanophore response to light and background in *Phoxinus* and the chameleon. Explanation in text.

slow to warrant such an interpretation (the transient expansion develops rapidly and subsides slowly over a period of about 10 min.). A similar transient expansion has been observed by Parker and Lanchester (1922) in *Fundulus*, and it is tempting to place great emphasis upon this additional parallelism between the chameleon and a fish, but it is hardly safe to do so until further evidence has come to light. It may be remarked, however, that apart from this one instance, no worker on fishes has had occasion to observe what happens to a fish immediately after it is removed from the dark and placed on a white background in the light. Such observations on *Phoxinus* would be of the greatest interest.

The experiment of Zoond and Bokenham with chameleons in light-proof jackets showed that on a white background the transient darkening did not occur, and the body of the animal remained in the condition of maximum pallor charac-

teristic of the animal equilibrated in darkness. The melanophore expansion in animals adapted to a black background, however, did occur whether the body was exposed to incident light or not. This shows that the white background response in chameleons is caused by the simultaneous stimulation of retinal and dermal photoreceptors, whereas the black background response can be evoked solely by the appropriate stimulation of the retina. The data do not at present exist for deciding whether the same conclusions apply to *Phoxinus*, or any other fish. It would be necessary to observe fishes in which the top of the head had been covered with some light-proof material, when they are removed from darkness to a white background in light.

Fig. 3 is a scheme which summarises the mechanism of co-ordination that has been developed above. The autonomic discharge E contracts the melanophores. The afferent impulses a, from the skin of chameleons or from the parietal organ in *Phoxinus*, inhibit this discharge. The afferent discharge b, from the retina resulting from black background stimulation, reinforces the effect of a. The afferent discharge w, from the retina in an environment with a white background, suppresses the inhibiting influence of a on E.

The nervous activity involved in these responses may be summarised in tabular form as follows:

Conditions	Activity in the nervous system	Melanophore response
Darkness	E	Contraction
Blind animal in light	$E - a$	Expansion
Normal animal on black background	$E - (a + b)$	Expansion
Normal animal on white background	$(a - w) + E$	Contraction

(*Note.* The minus sign is here employed to denote inhibition.)

The above considerations are clearly inapplicable to those chromatically active fishes (and reptiles, if any) which do not become pale in darkness, and which, when blinded, do not darken when exposed to the light.

In connection with the photosensitivity of blind minnows it is interesting to note the remarkable observations of Scharrer (1928) who induced feeding responses to light in such animals by the method of conditioned reflexes.

(7) *"Psychic" response.*

Long before the physiological study of colour response began, chameleons were popularly supposed to be highly emotional animals, and to display their feelings by vivid changes in skin pigmentation. This conception was probably an anthropomorphically biased interpretation of the phenomenon of colour change. Brücke (1852) observed that when he handled a chameleon roughly the animal turned dark in colour. Pouchet (1876) found that excitement and fright caused the appearance of dark spots on a pale-adapted turbot. Sumner (1911) also noticed that his flounders when disturbed became darker, and Schaefer (1921) records the same for *Pleuronectes*. Von Frisch (1912) has discussed the phenomenon. He did not find that

emotional excitation caused darkening in all cases. The gurnard (*Trigla lineata*), which is normally bright red, turned white, and *Phoxinus* also became pale. The stingfish (*Scorpaena porcus*), on the other hand, became darker. Giersberg (1932) observed that in *Phoxinus* this form of stimulation induced expansion of the coloured chromatophores.

In certain fishes a characteristic coloration appears during the breeding season. It has been investigated by Becher (1924), Wunder (1931) and Osterhage (1932). The last-named has described the appearance of the " Hochzeitskleid " in *Rhodeus amarus*. A bright golden red pigmentation develops on the operculum and the ventral surface of the body, and red pigment also appears in the iris and the fins. Becher is of the opinion that this is due to a proliferation of red chromatophores in the skin, in which case the phenomenon is not a true physiological colour response, but belongs to the category of morphological colour change. (On this subject see Cunningham, 1891; Mayerhofer, 1909; Kuntz, 1915; Kudo, 1922.)

Few exact observations of emotional colour change appear to have been made on reptiles. Hadley (1929) described the copulation of the lizard (*Anolis porcatus*). While performing the antics of courtship the male is light coloured. " At the onset of copulation the female is usually brown and remains brown throughout, while the male is usually green for the first few minutes of copulation, turns brown and remains in the dark state for the remainder of the copulation time, in some cases more than an hour." It is difficult to assess the period of most intense emotional excitation during this cycle of events. Hadley also remarks that " Alarm, jealousy or the sexual instinct cause an animal to assume the light state, thus masking the light and temperature effects."

I have carried out some unpublished observations on the Cape chameleon (*Lophosaura pumila*). Chameleons were excited by pinching their feet with a pair of forceps. This caused them to hiss noisily with mouth wide open and to attempt to bite. They frequently behave in this manner towards one another. Forceps' stimulation of animals adapted to white background induced darkening of the skin after 1 or 2 min. The same happened when a pale animal was stimulated in the dark. Forceps' stimulation of dark animals on a black background induced no change in the shade of the skin, though, of course, the hissing and biting occurred as usual. It has already been pointed out that the term "excitement pallor" employed by Hogben and Mirvish to describe the melanophore contraction which results from electrical stimulation of the roof of the mouth in the chameleon is inappropriate. Electrical stimulation is not excitement, and excitement causes, it would seem, not pallor but darkening.

The evidence, for comparative purposes, is meagre, but there is enough to indicate that in both fishes and reptiles escape and defence reactions are frequently associated with expansion of the melanophores.

(8) *Contraction of denervated melanophores.*

Several authors have observed that, although section of nerves causes paralysis of the corresponding melanophores, sooner or later signs of activity begin to appear.

Pouchet (1876) stated that "ces régions paralysées peuvent se montrer tantôt plus foncées, tantôt plus pâles que le reste de l'animal." Von Frisch (1911 c) noted that when the sympathetic was destroyed the contrast between the normal and paralysed regions of the skin vanished after 13 days. Moreover, the denervated melanophores responded by contraction to emotional excitation 2 or 3 days after the operation. Smith (1931) has followed this up, and has extended von Frisch's experiments on *Phoxinus*. He showed that, after cutting the ophthalmic branch of the trigeminal, the denervated region becomes pale after several days if the fishes are kept on a white background. Since this does not happen on a black background, it must be inferred that some non-nervous mechanism exists which serves to co-ordinate the adaptive response of denervated melanophores. The same phenomenon has been investigated by Mills (1932 b) in *Fundulus*. She observed that responses in a denervated region start at the periphery and are gradually transmitted to the centre, and this is advanced as evidence for a neurohumoral control of fish melanophores (see p. 368). Parker (1934 b) has recorded the time taken for the denervated strip to respond to a dark background. The whole fish darkened in 1·8 hours, a strip 1 mm. in width darkened in 20·5 hours. Smith (1931) also confirmed von Frisch's observation that "frightening" a fish induced immediate and rapidly reversible contraction in the denervated region.

Few observations of this nature appear to have been recorded for reptiles. The puzzling statement of Redfield (1918) that "when the nerves supplying a region of the skin are cut (in *Phrynosoma*), and the surface of the mouth or cloaca is stimulated..., the melanophore pigment of the entire surface of the skin contracts," may perhaps find its explanation in the observation of Zoond and Eyre that in the chameleon any locus on the skin is supplied by two consecutive spinal nerves.

In chameleons I have noticed that after a number of days the melanophores in a denervated region begin to show contraction (unpublished observations). There is some indication also that when this occurs the denervated melanophores regain to some extent their power of adaptive colour response, but the observations are not sufficiently definite to warrant further discussion.

All that can at present be said is that in both fishes and reptiles non-nervous agencies most probably exist which play their part in normal chromatic responses. They are masked by the dominant role of the nervous system, but further study of their nature and mode of action may well supply information of fundamental importance to comparative physiology.

III. THE COLOURED CHROMATOPHORES.

In addition to melanophores, the chromatic equipment of vertebrates comprises cells containing yellow and red pigment, which, unlike the melanophore pigment, is soluble in lipoid solvents. There are also the guanophores or iridocytes containing minute guanin crystals. These cells, when present in sufficient numbers, constitute the argenteum of fishes (Cunningham and MacMunn, 1893).

The anatomical relations of these elements in reptilian skin have been described by Keller (1895), Schmidt (1918) and von Geldern (1921). It is not known with

certainty whether they play a dynamic or merely static role in the pigmentary response of reptiles. The nature of reptilian skin is such that these cells can only be examined in microscopic section; they do not lend themselves to direct observation in the living state, such as is possible in fishes. It appears that, in spite of the bright pigmentation of many species, no colour changes have ever been observed in reptiles which really indicate the functional activity of coloured chromatophores. For this reason the discussion of the physiology of these elements must be limited to fishes.

(1) *Adaptation to coloured backgrounds.*

The adaptation of *Phoxinus* to backgrounds of different colours was studied by von Frisch (1912). Red and yellow backgrounds both caused a combined expansion of the yellow and red pigment cells, but on other colours these cells remained unaffected (contracted), and only the melanophore response occurred. Von Frisch's careful experiments demonstrated beyond doubt that the expansion of the coloured cells was a response to the colour and not merely to the shade of the background. The independent activity of the melanophores as against the xantho- and erythrophores was shown by transferring yellow-adapted animals some to white and some to black. In the former the yellow and red cells contracted and the melanophores remained in the contracted state. In the latter the yellow and red cells also contracted, but the melanophores expanded. The flounders investigated by Mast (1914), on the other hand, showed a greater range of colour adaptations. Yellow, red, green and blue all evoked a corresponding shade in the fish, though only after a very prolonged exposure, 2 or 3 months. The greens and blues were due apparently to a change in the relations of the guanophores, and it is not unlikely that these responses were of the developmental rather than physiological type. Connolly (1925) and Fries (1927) confirmed, in *Fundulus*, the findings of von Frisch. Meyer (1931), working on *Gobius*, is of the opinion that a difference in the responses of xanthophores and erythrophores to yellow and red background can be detected in this fish.

(2) *Control of the coloured chromatophores.*

Adaptation to coloured backgrounds is abolished by blinding. Whether in blinded fishes the xantho- and erythrophores still respond to light and darkness has not, apparently, been recorded, but it is probable that they do not. If this is so, then the behaviour of the coloured chromatophores is less complicated than that of the melanophores. Von Frisch (1912) was able to demonstrate in *Trigla* and *Crenilabrus* that essentially the same system of nerve paths and connections supplies the lipophores as the melanophores. He performed on these fishes most of the experiments on nerve cutting and brain stimulation which had served for the analysis of melanophore control in *Phoxinus*. In this fish, however, the control of the coloured chromatophores appears to be quite different, for Giersberg (1930) has brought forward evidence to show that they are not innervated at all, and that, in consequence, adrenalin, which invariably exerts a pharmacodynamic effect on innervated pigmentary effectors, has no influence upon them. On the other hand,

pituitary extract causes their maximal expansion. In a later paper, Giersberg (1932) extended his observations on the role of the hypophysis in the activity of the xantho- and erythrophores (lipophores) of the minnow. Destruction of the hypophysis resulted in contraction of the lipophores, and injection of infundin, or implantation of a hypophysis in the body cavity, caused an expansion which lasted some hours, and was independent of environmental conditions. This effect of infundin injection on the lipophores of *Phoxinus* has been reported also by Abolin (1924). The work of Abolin and Giersberg appears to furnish clear evidence of the control of colour adaptation in the minnow by endocrine agencies, but the whole issue becomes confused when we turn to the observations of Meyer (1931). She found that it was possible to induce *melanophore* contraction and expansion in soles and flounders by the injection of the centrifuged serum from white-adapted and black-adapted fishes respectively. Now there can be little doubt that the melanophores of the *Pleuronectides* are under nervous control. Meyer's results suggest that an endocrine mechanism also exists. On the other hand Matthews (1933) found that hypophysectomised *Fundulus* responded normally to white and black backgrounds. The experiments of Osterhage (1932) add to the confusion. Injection of hypophysial extract into the stickleback (*Gasterosteus*) and into *Rhodeus* caused expansion of the melanophores, but had no effect on the lipophores. He tried out the effect of a large number of tissue extracts and other substances, and showed that a fair proportion of them had an effect on the melanophores. Melanophore expansion was evoked by hypophysin, testicular extract, atropin, johimbin, physiological and saturated salt solution, and tyrosin. Contraction was caused by adrenalin, testiglandol, testis-opton, and potassium chloride. The importance of these observations resides in the fact that they demonstrate the sensitivity of pigment cells to various physiological substances, and they emphasise the necessity for caution in the theoretical interpretation of such data. Osterhage did not claim to have proved that the colour response of his fishes is controlled by the adrenal organs, the pituitary and the gonads; yet a number of workers who have injected only one or two substances have constructed theories on the basis of their results.

The results obtained by several authors with the injection of adrenal and pituitary hormones into various fishes are summarised in Table II.

Table II. *Effect of endocrines on the chromatophores of fishes.*

Author	Fish	Adrenalin		Pituitrin	
		Melano-phore	Lipo-phore	Melano-phore	Lipo-phore
Hewer (1927)	*Phoxinus*	+	+	+	−
Wyman (1924)	*Fundulus*	+	.	+	.
Abolin (1924)	*Phoxinus*	+	o	.	−
Giersberg (1932)	*Phoxinus*	.	o	.	−
Osterhage (1932)	*Gasterosteus*	+	.	−	.

+ denotes contraction; − expansion; o, no effect.

IV. CONCLUSION.

The literature of colour response in fishes and reptiles does not yield a body of facts which readily lends itself to a general synthesis. Hitherto, no attempt has been made at a complete formulation of the physiological processes which co-ordinate the pigmentary activity of these two groups. The interrelation of the reflex responses to direct illumination of the body surface and to the photic properties of the background has not been considered, and the physiological processes involved have been usually summed up in some such vague phrase as "a direct nerve control supplemented by a humoral influence." There is, of course, no justification for the assumption that the physiological mechanism of pigmentary control is identical or even similar within the limits of a single class of animals, and still less when two classes are compared with each other. But when account is taken of the striking correspondence in the anatomical units concerned in colour change, and of the many points of identity in the bionomic aspects of the phenomenon, it appears extremely unlikely that there should not be also a fundamental identity in the physiological co-ordinating apparatus. The conception of nerve control supplemented by humoral influence is most probably accurate so far as it goes. But it does not go far enough. In the present review it has been shown that when the data for the minnow and the chameleon are considered it is possible to formulate a theory of co-ordination of *melanophore* activity which fits them both. The data for other fishes and reptiles do not permit of a more general application of the theory, though in a number of cases observations have been recorded which are in agreement with it. It is perhaps permissible to remark, without incurring the stigma of partisanship, that the minnow and the chameleon have been investigated by a greater number of workers than other species of fishes or reptiles, with the possible exception of *Fundulus*. It is also noteworthy that those species which present the greatest deviation from the minnow and chameleon mechanism have been examined only with reference to one or two aspects of their chromatic behaviour. Thus there is evidence that the melanophores of *Anolis equestris* function as independent effectors in relation to light and heat stimuli, but data on the effect of nerve section and stimulation, and on the background responses of this animal do not exist. The same may be said of the catfish (*Amiurus*), whose melanophores are said to contract in the light and expand in the dark (Bray, 1918). Such information for these two animals would be of the greatest interest, and would furnish the materials for comparisons of fundamental importance.

The physiology of the lipophores of reptiles is almost an unexplored field, and consequently any attempt at a comparison with fishes is out of the question. In fishes there is little to suggest that the lipophores are influenced by *photic* stimuli other than those that affect the eyes and depend upon the colour of the background. On the other hand, there is a possibility that emotional states and periodic sexual phenomena may affect the lipophores to a marked degree. In view of the conflicting evidence it is not possible at present to draw any general conclusions, but there can be little doubt that the pituitary gland is to some extent concerned in these pro-

cesses. There are also indications that the *melanophores* of fishes are not entirely unaffected by endocrine agencies. In this connection it is important to note that all the fishes dealt with by the investigators cited in this article were teleosts.

The pigmentary physiology of elasmobranch fishes appears to be quite different. Lundstrom and Bard (1932) found that in the dogfish (*Mustelis canis*) hypophysectomy produced a maximal contraction of the melanophores, and injections of "pituitrin" and "infundin" into such animals caused darkening. Parker and Porter (1934) observed background adaptation in this animal (recorded also in the skate (*Raja erinacea*) by Parker, 1933), and further arrived at the conclusion that in addition to the pituitary principle which promotes melanophore expansion, there is a nervous mechanism which controls contraction. This conclusion, however, was based on the belief that transection of nerves causes their permanent excitation (see p. 367), and would have been more convincing if it had been shown that electrical stimulation of these nerves in the dogfish evoked the same response as their transection, namely, melanophore contraction. In this context it should be noted that Young (1933) has reported the absence of grey rami in Selachians, and has inferred, therefore, that their chromatophores are not under nervous control.

These results, though incomplete, suggest a resemblance to amphibian colour response, and certainly do not permit of any comparison with teleosts.

V. SUMMARY.

1. There is evidence that in both reptiles and teleost fishes the melanophores react to light in two ways:

(*a*) Expansion in response to illumination of the body surface or some part of it.

(*b*) Adaptation to shade of background.

2. Both processes must be considered in the formulation of a theory of co-ordination of pigmentary response.

3. Such a theory is discussed, and is shown to be applicable to the minnow and the chameleon.

4. The lipophores are concerned in adaptation of fishes to the colour of the background.

5. Recent work shows that they are controlled by endocrine agencies, which may also influence the melanophores to some extent.

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DIE BIOELEKTRISCHEN ERSCHEINUNGEN ARCHITEKTONISCHER FELDER DER GROSSHIRNRINDE[1]

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INHALT.

I. EINLEITUNG.

(1) DIE ARTEN DER HIRNSTRÖME.

DIE *normale* Hirnrinde produziert Ströme, auch wenn alle Sinnesreize so gut wie möglich ausgeschaltet sind und das Versuchstier keinerlei effektorische Leistungen, wie Bewegungen und Drüsensekretion, erkennen lässt. Diesen *ständig vorhandenen Strömen,* die als *Feldeigenströme* (*FES*) (Kornmüller, 1933 e) bezeichnet werden, kann man die *Aktionsströme* gegenüberstellen. Letztere treten auf Aussenreize hin auf und sind zeitlich streng an diese gebunden.

[1] Meinen lieben Eltern in Dankbarkeit gewidmet.

Sind die Versuchsbedingungen oder der Zustand des Tieres *nicht normal,* so treten *abnorme Stromabläufe* auf der Hirnrinde in Erscheinung. Abnorme Zustände des Gehirns können mit vielgestaltigen Übergängen zu extremer Abänderung des bioelektrischen Normalbildes, sowohl zum *völligen Verschwinden aller elektrischen Spannungsproduktionen* als auch zu *enormen Steigerungen* derselben, führen. Letztere bezeichnen wir als *Krampfströme (KS).* Sie bilden sich auf Grundlage einer starken Übererregbarkeit der Hirnrinde und können motorischen Krämpfen zeitlich entsprechen.

(2) Historisches.

Es bleibt erstaunlich, dass, als wir Anfang 1931 an das Studium der bioelektrischen Erscheinungen der Grosshirnrinde herangingen, die Tatsache allgemein wenig bekannt war, dass das Gehirn lokalisierbare Spannungsproduktionen zeigt, und dass den wenigen Beobachtungen älterer Autoren allergrösste Skepsis entgegengebracht wurde. Es ist bemerkenswert, dass man selbst in den Handbüchern der Jahre zuvor, wie auch in anderen zusammenfassenden Darstellungen, nicht einmal Andeutungen von der Möglichkeit der Ableitung bioelektrischer Erscheinungen der Grosshirnrinde findet.

Die *ständig vorhandenen Ströme* und deren Lokalisation, die das meiste Interesse beanspruchen dürfen, fanden bisher keine systematische Bearbeitung. Wir haben diesen ausgedehnte Untersuchungen gewidmet (Kornmüller, 1932*b*, 1933*a* und *e*, 1934).

Der erste, der die Beobachtung machte, dass das Gehirn auf peripheren Sinnesreiz eine lokalisierte negative Schwankung zeigt, war der englische Arzt Caton (1875). Unabhängig von diesem konnte 1890 die gleiche Beobachtung von Fleischl von Marxow (1893) und von A. Beck (1890) gemacht werden. Die Hoffnungen der genannten Autoren, mittels der negativen Schwankungen Lokalisation auf der Grosshirnrinde zu treiben, wurden aber auch durch deren Nachuntersucher (Gotch und Horsley, 1891; Právdicz-Neminski, 1913; u. a.) keinesfalls erfüllt. Das Interesse an solchen Untersuchungen hörte vollends auf. Jahrelang erschien keine Arbeit auf diesem Gebiete, so dass die alten Untersuchungen in Vergessenheit gerieten, beziehungsweise mit grösster Skepsis betrachtet wurden. Schliesslich konnte der Verfasser (1932–34) durch Ausnützung der inzwischen weit vorgeschrittenen Technik und vor allem durch die Verknüpfung mit der Hirnrindenarchitektonik aufzeigen, welch ungeahnten Wert die bioelektrische Methode für das Lokalisationsproblem hat. An Hand von Aktionsströmen an Säugetiergehirnen haben über rein physiologische Fragestellungen einige Autoren in den letzten Jahren gearbeitet. Zu erwähnen sind Bartley (1933*a*, 1933*b*), Bartley und Newman (1931), Bishop und Bartley (1932), Fischer (1932, 1933), Gerard, Marshall und Saul (1933), Perkins (1933), u.a. Da meine Untersuchungen und in gleicher Weise die meines engsten Mitarbeiters Tönnies (1932–34), was Methodik und Fragestellungen betrifft, nur wenige Beziehungen zu den Arbeiten der genannten Autoren haben, sei der Kürze halber hier noch auf das Literaturverzeichnis hingewiesen. Auf einzelne Arbeiten kommen wir weiter unten zu sprechen. Es muss noch besonders darauf hingewiesen werden, dass sich auch Adrian und Matthews (1934)

in neuester Zeit mit dem Studium des Wesens der bioelektrischen Erscheinungen der Grosshirnrinde beschäftigen. Durch unsere Untersuchungen angeregt hat Wang (1934) einen Beitrag über die Aktionsströme der Sehrinde und der Vierhügel bei Lichtreiz geliefert und ausserdem Satô (1933) eine Methode der Lokalisation auf der Grosshirnrinde durch Widerstandsmessung angegeben.

Besonders erwähnt sei hier das "Elektrenkephalogramm" (*EEG*), jene elektrischen Erscheinungen, die Berger (1929–34) vom uneröffneten menschlichen Hirnschädel ableitet. Das *EEG* ergab bis jetzt keinerlei lokalisatorische Ergebnisse. Darauf kommen wir im Abschnitt über die bioelektrischen Erscheinungen des menschlichen Hirns zu sprechen. Über das Elektrenkephalogramm liegen ausserdem neuere Untersuchungen von Tönnies (1933 *b*, 1934 *b*) und Adrian vor.

Die ersten Untersuchungen über abnorme Stromabläufe wurden von Kornmüller (Vortrag 1932 (1933 *a*)) und von Fischer (1933) angestellt.

(3) GRUNDTATSACHEN ÜBER DEN FEINEREN BAU DER HIRNRINDE (ARCHITEKTONIK).

Unsere Studien unterscheiden sich von denen der anderen Autoren vor allem auch dadurch, dass wir *von der Morphologie ausgehen und die Tatsachen der Hirnrindenarchitektonik zur Grundlage unserer physiologischen Untersuchungen machen.*

Durch Campbell, Elliot Smith, insbesondere die Schule von O. Vogt (1907, 1919) mit K. Brodmann (1909) und anderen wurde aufgezeigt, dass die Hirnrinde der Säugetiere und des Menschen nicht einheitlich in ihrem Bau ist, sondern dass man auf der Hirnrinde eine grosse Zahl von Feldern unterscheiden kann, die sich durch ihren Bau voneinander unterscheiden, die *Areae architectonicae*. Ausser den genannten Autoren haben sich vor allem Mauss, C. Vogt, v. Economo und Koskinas, Rose (1931) und E. Beck um die Kenntnisse über die Architektonik der Hirnrinde verdient gemacht. Darüber hinaus konnten C. u. O. Vogt (1907, 1919) den Wert der architektonischen Tatsachen für die Physiologie an Hand ihrer grundlegenden und umfassenden Hirnrindenreizungen aufzeigen.

Fig. 1 zeigt oben einen Schnitt der Hirnrinde des Affen bei 40-facher Vergrösserung. Zum besseren Verständnis dieser Mikrophotographie ist deren mittlerer Ausschnitt unten in Fig. 1 *b*[1] schematisch dargestellt. Das Schema (Fig. 1 *b*) zeigt in vergröberter Form die mikroskopischen Befunde von Fig. 1 *a*. Die Ganglienzellen sieht man deutlich in *Schichten* parallel zur freien Oberfläche angeordnet. Die Schichten sind seitlich eingezeichnet. An der mit Pfeil und bei Fig. 1 *a* ausserdem mit einem künstlichen Einstich bezeichneten Stelle treten ganz plötzlich Modifikationen im Bau aller Schichten auf. Links vom Pfeil sieht man in beiden Bildern die *Area* 17 (*Striata*), rechts die *Area* 18. Was die Schichtenbreite betrifft, ist rechts die III-Schicht ebenso wie die V-Schicht breiter als links, die IV-Schicht dagegen ist links um vieles breiter als auf der anderen Seite. Die III- und VI-Schichten sind rechts merklich zellärmer als links. Schon bei dieser schwachen Vergrösserung kann man Unterschiede in der Zellgrösse, Zellform und -anordnung sehen. In der

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[1] Fig. 1 *b* stammt aus einer Arbeit von O. Vogt (1928).

III-Schicht der *Area* 18 sind die Pyramidenzellen grösser als in der der *Area* 17. Ganz besonders bemerkenswert ist die Tatsache, dass *die IV-Schicht links bei einer*
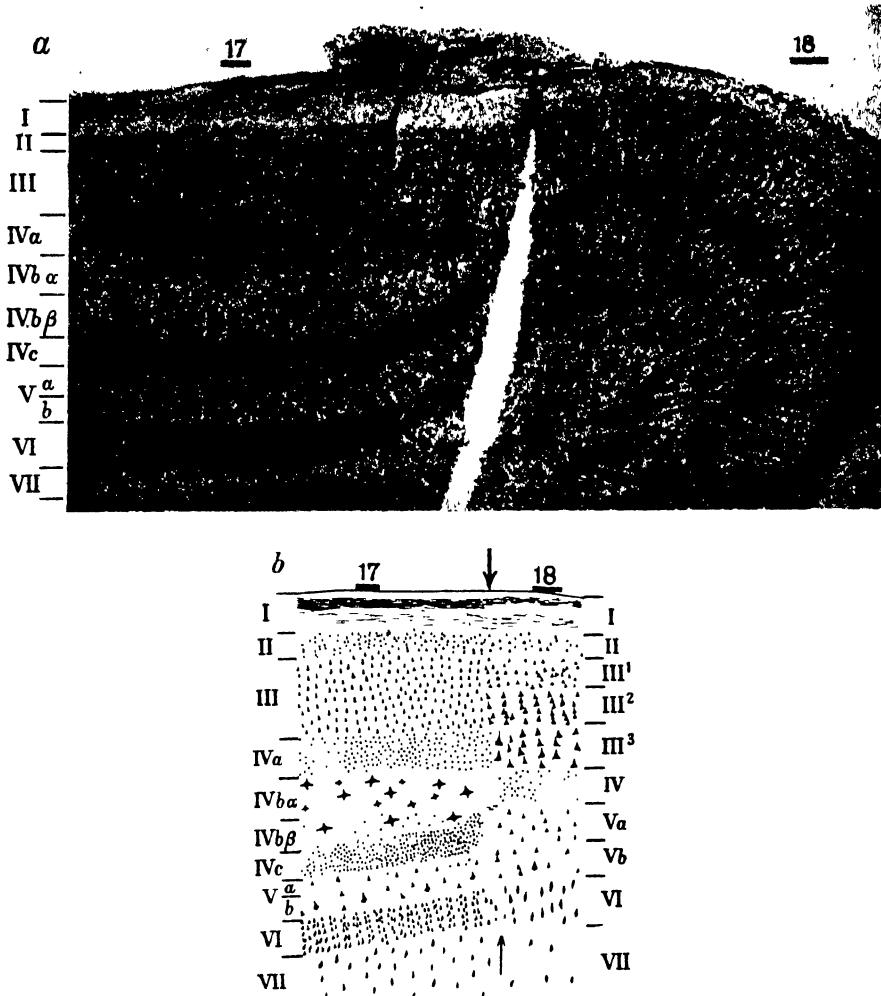
Fig. 1. *a*, Mikroskopisches Bild einer Hirnrindenstelle des Affen (*Cynomolgus*). Horizontalschnitt des Okzipitalhirns. Zellfärbung. Vergrösserung 1 : 40. *b*, Schematische Darstellung des mikroskopischen Befundes von 1 *a*. Der Pfeil markiert die architektonische Grenze zwischen *Area* 17 (links) und *Area* 18 (rechts). Seitlich sind die Zellschichten der genannten Felder eingezeichnet. Der Einstich in der Mitte des oberen Bildes charakterisiert eine Grenze zwischen Teilen der Hirnrinde, die in ihren bioelektrischen Erscheinungen eindeutige Unterschiede aufweisen. Koinzidenz der bioelektrischen mit der architektonischen Grenze.

vielfach grösseren Breite deutlich eine Reihe von Unterschichten erkennen lässt, während die schmale IV-Schicht der *Area* 18 einheitlich erscheint und keine Unterschichten abgrenzen lässt.

Wenn auch bei weitem nicht alle architektonischen Grenzen so augenfällig sind wie die beschriebene, so sind sie doch deutlich erkennbar. So wurden vermittels des Mikroskopes *Gliederungen der Hirnrinde durch Abgrenzung ungleich gebauter Areale* vorgenommen. Die Bauunterschiede der einzelnen Hirnrindenfelder beziehen sich nicht nur auf Differenzen in der Zahl, der Anordnung und der gröberen Form der Zellen, sondern auch der Markfasern. Auf Fig. 2 ist oben als ein Beispiel die Gliederung der Hirnrinde des Kaninchens in architektonische Felder nach M. Rose (1931) zu sehen.

II. METHODIK.

Einzelheiten über die Methodik sind in unseren früheren Arbeiten (Kornmüller, 1932 *b*, 1933 *a*, *d*, *e*) und über deren physikalisch-technischen Teil in den Mitteilungen von Tönnies (1932, 1933 *a*, *b*) niedergelegt. In Kürze seien darum hier nur die wichtigsten Punkte angedeutet.

Da die normalen bioelektrischen Erscheinungen der Grosshirnrinde sehr leicht alterierbar sind, ist es notwendig, den *operativen Eingriff* so *gering und schonend* wie nur möglich zu gestalten.

Im Gegensatz zu denen der anderen Autoren erfolgten unsere Ableitungen vom Gehirn *unipolar*; diese Art der Ableitung schien mir von Anfang an die geeignetste für lokalisatorische Fragestellungen zu sein. *Die indifferente Elektrode wird an das blossliegende Nasenbein oder an eine andere Stelle des Kopfes, die sich nach einer Prüfung als frei von Potentialschwankungen erweist, gelegt.* Nur die *differente Elektrode* liegt auf der zu untersuchenden Hirnstelle, so dass die zur Registrierung gelangenden Potentialschwankungen nur von dieser einen Stelle stammen können.

Zur Registrierung verwendeten wir folgende Apparaturen:

(1) Den von Tönnies (1932, 1933 *a*) in unserem Institut entwickelten und konstruierten Neurographen. Dieser Apparat besteht aus einem Gleichstromverstärker, der eine Spannungsverstärkung auf das 10-Millionenfache ermöglicht, und aus einem Registriergerät, das mit einer tintegefüllten Schreibfeder die Spannungsschwankungen in unmittelbar sichtbarer Schrift auf einen 45 mm breiten Streifen gewöhnlichen Schreibpapiers aufzeichnet. Die Dämpfung und die Eigenschwingung des Schreibsystems liegen genügend hoch, um fast alle auf der Hirnrinde vorkommenden Spannungsschwankungen naturgetreu wiederzugeben. Dieser Apparat lässt während *eines* Experimentes ohne grossen Kostenaufwand viele hundert Meter Kurven gewinnen, die bei dem variablen Bild der bioelektrischen Erscheinungen des Gehirns zur Erkennung von Gesetzmässigkeiten von grossem Nutzen sind. Nach dem gleichen Prinzip wurde ausserdem der Polyneurograph gebaut, welcher die *gleichzeitige Registrierung* der Potentialschwankungen von fünf verschiedenen Stellen des Zentralnervensystems mit eben so vielen Schreibfedern erlaubt, wodurch sich in erster Linie die Wechselbeziehungen zwischen den verschiedenen Teilen des Zentralnervensystems feststellen lassen.

(2) Ein *Einthoven*sches Saitengalvanometer in Zusammenarbeit mit einem Gleichstromverstärker.

(3) Eine Oszillographenschleife mit 3000 Hertz Eigenschwingungszahl, die an die Endstufe eines Neurographenverstärkers angeschlossen ist.

Die Ableitestellen wurden während des Experimentes in eine detaillierte Skizze der Hirnoberfläche, in welcher die Gefässe der weichen Hirnhaut als Anhaltspunkte eingezeichnet wurden, eingetragen und auf Grund dieser am Ende des Experimentes durch einen feinen Einstich oder durch zartes Betupfen mit einem Kristall von Argentum nitricum markiert. Zur genauen Lokalisation der Ableitestellen wurden die architektonischen Rindenfelder verwendet. Dazu wurden die Hemisphären in Paraffin eingebettet, dann stets soweit wie nötig in lückenlosen Serien geschnitten und gefärbt.

III. ERGEBNISSE.

(1) ÜBER DAS LOKALISATIONSPROBLEM.

(a) Die ständig vorhandenen Ströme, Feldeigenströme.
Deren Typen und Lokalisation.

Die ersten Untersuchungen, die an Kaninchen angestellt wurden, galten der Frage, ob es möglich ist auf einen Sinnesreiz hin, z. B. Augenbelichtung, Aktionsströme abzuleiten, was auch bald gelang. Meist bauten sich die Aktionsströme auf eine gerade Linie auf, das heisst, es waren also weder vor noch nach denselben irgendwelche Schwankungen zu registrieren. Bald aber zeigten sich bei schonendem tierexperimentellen Vorgehen auch solche Schwankungen, deren bioelektrische Natur von mir bald erkannt wurde. Diese ständig vorhandenen Ströme, *Feldeigenströme*, sind am gesunden Gehirn stets vorhanden und häufig mit gleicher Amplitude wie die Aktionsströme. Wenn sie nicht zu registrieren sind, so ist dies nur ein Ausdruck dafür, dass das betreffende Gehirn geschädigt ist. Diese Ströme sind leichter zu beeinflussen als die Aktionsströme; sie verschwinden darum bei lähmenden Einwirkungen auf das Gehirn früher als die letzteren.

Die nächste Feststellung, die ich machen konnte, war die, dass die Feldeigenströme in ihrem Ablauf nicht gleichartig über dem ganzen Gehirn aussehen, sondern dass sich eine Reihe von Feldern auf der Grosshirnrinde unterscheiden lässt, von denen ein jedes einen anderen Typus des Feldeigenstromes aufweist. Fig. 2 stellt oben die Anordnung der architektonischen Felder des Kaninchenhirns nach Rose (1931) dar. Die Kurven darunter sind Registrierungen der Feldeigenströme, die von einer grossen Zahl der oben angegebenen Felder abgeleitet wurden. Jede Kurve trägt die Bezeichnung des Feldes, von dem sie registriert wurde. An den vielen daraufhin untersuchten Tieren haben wir, von geringen individuellen Differenzen abgesehen, immer wieder gleiche Kurven erhalten. Die *Papiergeschwindigkeit* war bei allen Registrierungen gleich, nämlich 33 *mm pro sec.*[1] Die Zeitmarkierung unter jeder Kurve schreibt Fünftelsekunden. Bei Betrachtung der Kurven fällt auf, dass häufig ähnliche Schwankungen serienweise folgen und man

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[1] Daraus lässt sich die *natürliche Grösse* der verkleinert reproduzierten Kurven der Figuren 5–12 und 2 bestimmen.

demnach eine Frequenz der Schwankungen angeben kann. Die Frequenz geben wir im folgenden in " Hertz " an, also der Zahl der Schwankungen pro Sekunde. Manche
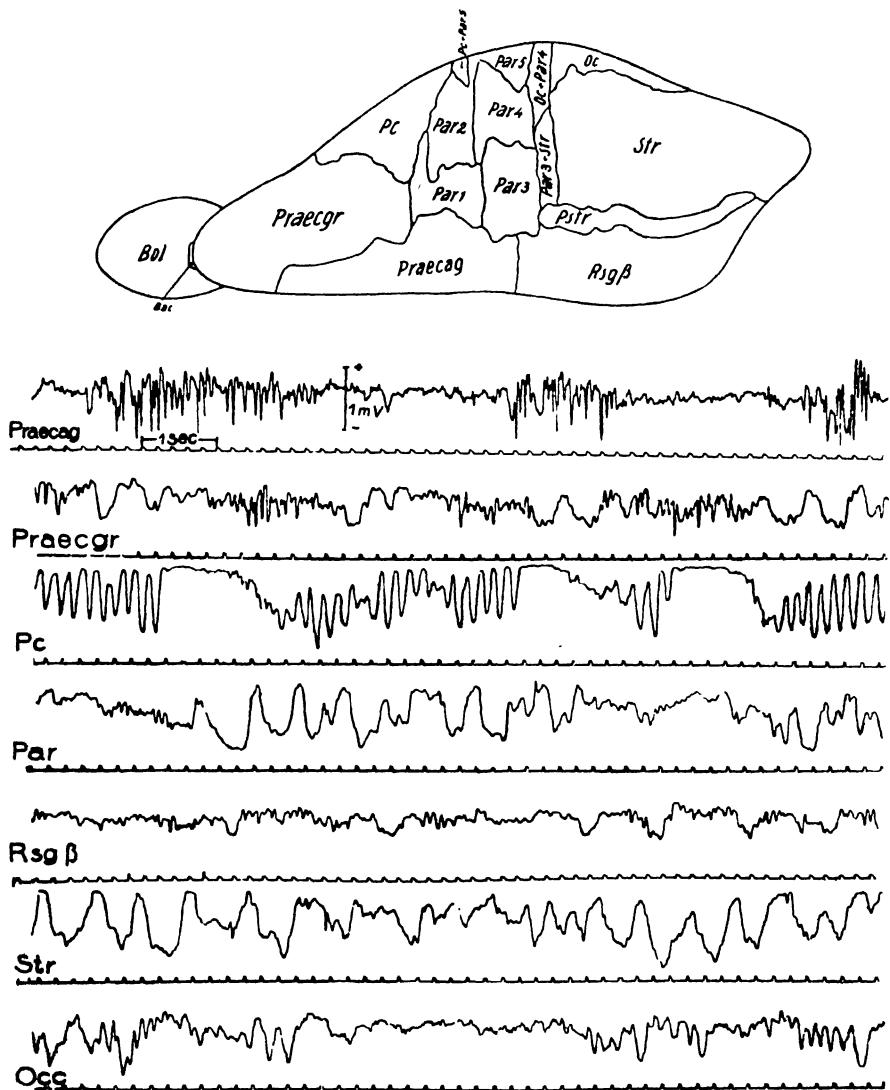
Fig. 2. Oben: die architektonische Felderung der Grosshirnrinde des Kaninchens nach Rose. Eine rechte Hemisphäre von oben gesehen. Darunter: die Normaltypen der Feldeigenströme. Registriert mit Tönnies' Neurographen.

Kurven zeigen im wesentlichen *eine* Hauptfrequenz (*Str* und *Praecag* der Fig. 2), andere wiederum mehrere, die sich meist periodisch abwechseln oder überlagern (*Praecgr*, *Pc* und *Par*). Wieder andere Kurven erscheinen wenig regelmässig, so dass

sich eine Grundfrequenz schwer angeben lässt (*Rsg β*). Bei diesen wollen wir die Zeitdauer der einzelnen Schwankungen in Sigmen angeben. Aus der Fig. 2 ist zu erkennen, dass ganz eindeutig *qualitative* Unterschiede zwischen den Kurven der einzelnen Felder bestehen. Daneben gibt es auch *quantitative* Differenzen. Es ist beispielsweise ein und dieselbe Frequenz in zwei Kurven verschiedener Felder vorhanden, doch die Amplituden sind immer in ihren Durchschnittswerten verschieden gross.

Die in Fig. 2 dargestellten Kurven seien im folgenden beschrieben:

Kurve *Praecag* wurde von der *Area praecentralis agranularis (Praecag)* abgeleitet. Sie zeigt periodische, häufig in unregelmässigen Abständen und mit ungleicher Amplitude wiederkehrende Gruppen von Abläufen mit durchschnittlich 15 Hertz Frequenz.[1] Letztere können wie fast alle *FES* des Kaninchens Spannungsproduktionen von 1 Millivolt und darüber aufweisen.

Um solche Kurven rein zu erhalten, muss sehr nahe oder ganz an der Mantelkante abgeleitet werden, da das architektonische Feld, das diese ergibt, sehr schmal ist und sich nur sehr wenig auf die Konvexität erstreckt. Neben diesen Wellen von 15 Hertz Frequenz konnten häufig auch noch Wellen wesentlich kleinerer Amplitude mit etwa 35 Hertz registriert werden.

Kurve *Praecgr* unterscheidet sich von der oben beschriebenen dadurch, dass sie neben den 15 Hertz-Wellen träge Schwankungen von etwa 3 Hertz aufweist, die sich mit den 15 Hertz-Schwankungen abwechseln.

Nebenbei bemerkt sind die beiden genannten Felder in ihrem architektonischen Bau sehr verwandt. Im wesentlichen unterscheiden sie sich nur dadurch voneinander, dass die *Area praecentralis granularis (Praecgr)* noch eine schmale Körnerschicht (IV-Schicht) besitzt, welche die *Area praecentralis agranularis (Praecag)* im Laufe der ontogenetischen Entwicklung verloren hat.

Kurve *Pc* stammt von der *Area postcentralis (Pc)* und ist durch einen periodischen Ablauf charakterisiert, der aus länger dauernden, etwa 7 Hertz-Wellen und aus etwa 13 Hertz-Wellen besteht. Letztere weisen auch geringere Amplituden auf. Bemerkenswert ist noch, dass die 13 Hertz-Wellen häufig auf der elektro-positiven Seite der Kurve begannen und allmählich auf die negative Seite hinübergingen, was auch die Abbildung zeigt. Es scheinen sich überhaupt rasche Abläufe mehr auf der positiven Seite zu finden. (Siehe z. B. auch die Kurven *Str* und *Occ*!)

Kurve *Par* wurde von einem *Parietalfeld (Par 1)* registriert. Dieses Feld zeigt Wellen von 15 Hertz ebenso wie die *Praecag*, doch von durchschnittlich geringerer Amplitude als letztere Area. Ausserdem liegen zwischen diesen Abläufen noch trägere Schwankungen von etwa 2–3 Hertz, welche zumeist von den ersten Abläufen überlagert sind.

Kurve *Rsg β* wurde von der *Area retrosplenialis granularis dorsalis (Rsg β)* abgeleitet. Sie zeigt keine ausgesprochene Periodizität. Grössere Amplituden weisen vereinzelt isolierte rasche Abläufe von etwa 50–70 Sigmen Zeitdauer auf.

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[1] An nicht kuraresierten Tieren sind nicht selten auch trägere Abläufe zu registrieren. Es zeigte sich aber, dass diese synchron mit der Atmung verliefen und darum wohl rein mechanisch durch Änderung der Elektrodenauflagefläche bei der Hirnpulsation zu erklären sind.

Daneben sieht man spärlichst kleine trägere Abläufe, die aber ebenfalls von den genannten raschen Schwankungen überlagert sind. Die abgebildete Kurve zeigt eine längere Strecke mit den Abläufen grösster Amplitude. Häufig aber zeigt dieses Feld über Sekunden nur ganz kleine Schwankungen, aus denen sich vereinzelte rasche Abläufe grösserer Amplitude herausheben.

Kurve *Str* zeigt den *FES* der *Area striata (Str)*, der vornehmlich aus trägen Abläufen besteht. Die mit grösserer Amplitude zeigen etwa 2 Hertz Frequenz und sind manchmal von sehr raschen Abläufen kleiner Amplitude überlagert.

Kurve *Occ* zeigt den *FES* der *Area occipitalis (Occ)*. Neben grossen Wellen von durchschnittlich 5–7 Hertz Frequenz sind kleinere Schwankungen von etwa 13–14 Hertz zu registrieren. Obgleich die Frequenzen denen der *Pc* ähnlich sind, so ist doch die Kurvenform durch andere Eigenheiten deutlich von der der *Pc* verschieden.

Durch die Frequenz und Amplitude allein sind die Kurven nicht eindeutig beschrieben. Das bezüglich Einzelheiten überaus wechselvolle Bild der Potential-schwankungen der Hirnrinde lässt sich überhaupt schwer exakt beschreiben. Auf andere Merkmale der Kurven, die wir (mit Tönnies, unveröffentlicht) bisher erfasst und zu analysieren versucht haben, wird später hingewiesen. Der Übersicht wegen wurden hier hauptsächlich die Abläufe mit grösserer Amplitude berücksichtigt. Nebenbei bemerkt sind wir in der Lage von sämtlichen Feldern der Hirnkarte der Fig. 2 den Typus der Feldeigenströme anzugeben. Davon wurde hier Abstand genommen.

Wie sind nun die einzelnen Typen der ständig vorhandenen Ströme des gesunden Gehirns gegeneinander abgegrenzt?

Leitet man unter den oben genannten Voraussetzungen mit einer differenten Elektrode ab, deren Auflagefläche auf dem Gehirn so klein wie nur möglich ist, so ist erstaunlich, dass plötzlich *nach einer kleinsten Verschiebung der Elektrode* schon ein anderer Typus zu registrieren ist. Die Grenze zwischen zwei verschiedenen Typen von Feldeigenströmen wurde auf der Hirnoberfläche durch feine Einstiche markiert. Die mikroskopische Untersuchung ergab in den sehr vielen untersuchten Fällen, dass der Einstich, der die bioelektrische Grenze markiert, mit einer archi-tektonischen Grenze koinzident ist. Siehe dazu Fig. 1, deren Beschreibung in den Abschnitten I, 3 und III, 1 *b* zu finden ist. Bei weit mehr als hundert untersuchten Gehirnen, konnte *immer wieder* dieses *Ergebnis* bestätigt werden, *dass* nämlich *die bioelektrischen Felder scharf begrenzt sind*, und *dass die bioelektrischen Grenzen mit architektonischen räumlich streng zusammenfallen*.[1]

Die grossen Differenzen in den Feldeigenströmen der Hirnrinde und deren scharfe Begrenzung können noch eindringlicher erwiesen werden durch gleichzeitige mehrfache Ableitungen, wie sie Tönnies in unserem Institut ermöglicht und gemeinsam mit mir durchgeführt hat.

Fig. 3 zeigt das Ergebnis einer gleichzeitigen Ableitung von zwei verschiedenen Stellen der *Area striata* des Kaninchens zu zwei Registrierapparaten (Oszillo-

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[1] Trotz alledem soll die Bezeichnung "*Feld*"-eigenstrom nur der Tatsache gerecht werden, dass die Eigenströme feldmässig lokalisiert sind, dass es also eine *bioelektrische* Felderung gibt. Es soll damit aus heuristischen Grunden nicht vorweggenommen werden, dass sich *in jedem Falle* die bioelektrischen Felder mit den architektonischen decken.
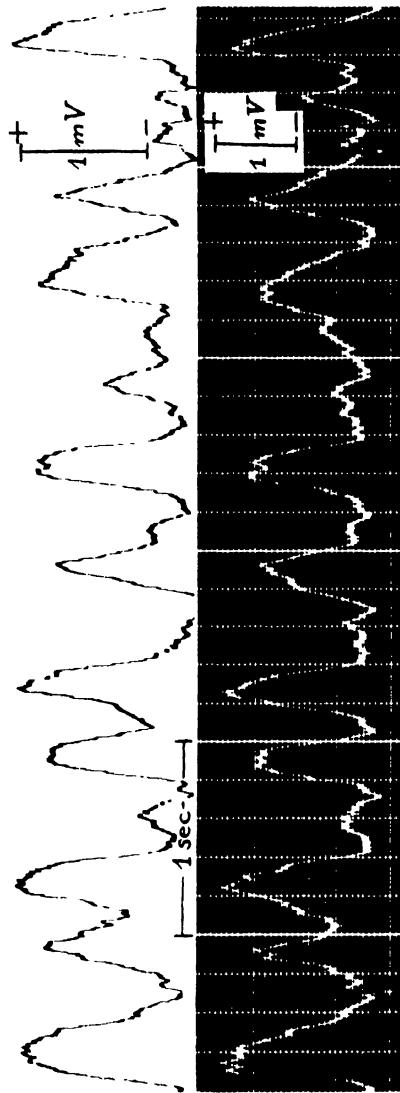
Fig. 3. Gleichzeitige Registrierung der Feldeigenstrome (*FES*) zweier Punkte ein und desselben architektonischen Feldes, der *Area striata* des Kaninchens. Der Abstand der beiden differenten Elektroden betrug etwa 1 cm. Auf den gleichen photographischen Streifen oben durch eine Oszillographenschleife und unten durch ein Einthoven-Galvanometer registriert. Die Potentialschwankungen sind weitgehend synchron.
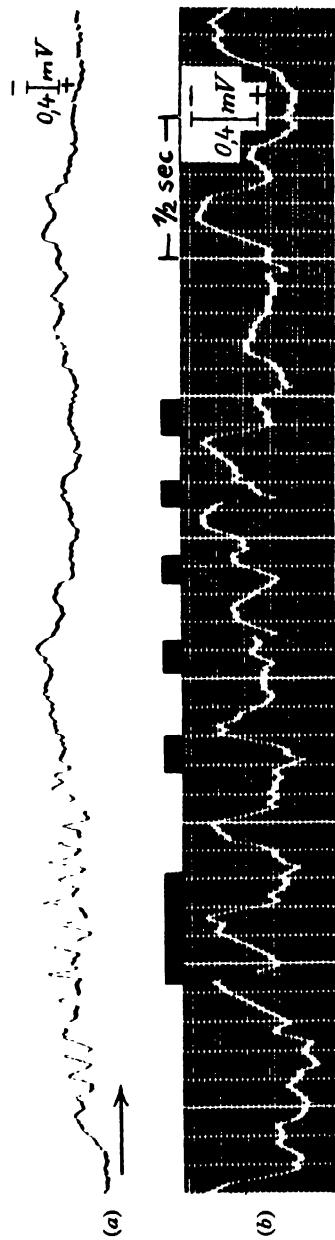
Fig. 4. Gleichzeitige Registrierung der normalen bioelektrischen Erscheinungen (*FES*) der *Area praecentralis agranularis* (oben) und der *Area striata* (unten). Die Reizmarkierung in der Mitte bedeutet Augenbelichtung. Diese hat nur auf die untere Kurve (*Area striata*) einen Einfluss. Registrierung wie bei Fig. 3. Die Potentialschwankungen sind eindeutig dyschron.

graph und Einthoven-Galvanometer mit Vorverstärkung), die völlig unabhängig voneinander arbeiteten. Selbstverständlich wurde ebenfalls *unipolar* unter Benutzung einer gemeinsamen indifferenten Elektrode, wie oben beschrieben, abgeleitet. Der Abstand der beiden Hirnstellen betrug etwa 1 cm. Auf eine streng zeitliche Koinzidenz ist bei diesen Registrierungen besonders geachtet worden. Wie die Figur zeigt, sind die bioelektrischen Spannungsproduktionen der beiden Stellen ein und desselben Feldes weitgehend parallel, also synchron. Bei genauerer Betrachtung fällt aber auf, dass der Beginn ähnlicher Schwankungen nicht streng gleichzeitig erfolgt. Es kann sogar bald die eine, bald die andere Stelle ein früheres Einsetzen der Spannungsproduktion aufweisen. Dies ist wohl der Ausdruck eines Hin- und Herpendelns des Erregungsablaufes über das genannte Feld. Weitere Einzelheiten dieser Verhältnisse werden uns wohl gleichzeitige Ableitungen von mehr als zwei Stellen eines Feldes ergeben. Grob betrachtet kann man aber ohne Zweifel feststellen, dass *die Stromschwankungen ein und desselben Feldes unter normalen Umständen am Kaninchen weitgehend synchron* verlaufen. Bei genauerer Betrachtung sieht man allerdings, dass analoge Schwankungen ungleich gross sein können, selbst wenn die ungleiche Empfindlichkeit der Apparaturen in Rücksicht gezogen wird. Auch zeigt der genauere Ablauf geringe Unterschiede auf. Der weitgehende Synchronismus konnte auch von vielen Feldern anderer Tiere festgestellt werden. Bestimmte Felder des Affen, z. B. die *Area* 4, zeigen einen verhältnismässig schwach ausgeprägten Synchronismus in den Potentialschwankungen verschiedener Punkte. Derartige Befunde finden später in einer Mitteilung über den Funktionsmechanismus der Hirnrinde eine Besprechung.

Lässt man nun eine Elektrode über diesem Feld und verschiebt die differente Elektrode des zweiten Registrierapparates um kleinste Strecken, so merkt man, dass plötzlich nach einer kleinsten Verschiebung die Schwankungen der beiden Ableitestellen nicht mehr synchron sondern dyschron verlaufen. Dieser Dyschronismus in den Schwankungen tritt dann auf, wenn die zweite Elektrode die architektonische Grenze des Ableitefeldes der ersten Elektrode überschritten hat. Fig. 4 zeigt eine gleichzeitige Ableitung von zwei verschiedenen Feldern, und zwar der *Area praecentralis agranularis* (a) und der *Area striata* (b). Auf demselben Papierstreifen wurde wiederum mit einer Oszillographenschleife und einem Einthoven-Galvanometer registriert. Die *Potentialschwankungen* sind eindeutig *dyschron*. Die Reizmarkierung in der Mitte der Figur gibt Augenbelichtung an. Diese findet nur über der *Area striata* bioelektrische Antwort. Siehe den folgenden Abschnitt! Wir haben sehr viel solcher gleichzeitigen Ableitungen an verschiedenen Tieren registriert. Sie führen, wie der in Fig. 4 gezeigte Befund, deutlich und klar vor Augen, dass es neben der morphologischen Differenzierung in architektonische Felder auch eine weitgehende physiologische Differenzierung der Hirnrinde gibt. In letzter Zeit konnten wir die ersten Registrierungen mit dem im Abschnitt "Methodik" erwähnten 5-fachen Neurographen an Kaninchen und Affen vornehmen. Diese führten uns die *physiologische Differenzierung der Hirnrinde* noch weitgehender und eindringlicher vor Augen.

Von einigen wenigen Feldern konnten wir ebenfalls durch mehrfache gleich-

zeitige Ableitung feststellen, dass sie miteinander in *Wechselbeziehung* stehen. Zu diesem Schluss glauben wir uns in allen den Fällen berechtigt, wo *ein strenger Synchronismus einzelner Gruppen von Spannungsschwankungen aus verschiedenen Feldern* zu registrieren war. Hier sei erwähnt, dass beispielsweise die 15 Hertz-Wellen der *Areae praecentralis agranularis* (*Praecag*) und *granularis* (*Praecgr*) zeitlich weitgehend koinzident ablaufen. Gewisse Synchronismen zeigen z. B. auch die *Areae* 1 und 2 des Affen.

Im Hinblick auf eine Vertiefung unserer Kenntnisse über die bioelektrischen Erscheinungen der Grosshirnrinde haben wir die längste Zeit nicht gleichzeitig Untersuchungen an verschiedenen Tierarten angestellt, sondern fast ausschliesslich das Kaninchen als Versuchstier beibehalten. Wir haben bei weitem noch nicht alle unsere Beobachtungen, die wir an diesem Tier machen konnten, veröffentlicht, sondern uns in den bisherigen Mitteilungen lediglich auf die Befunde beschränkt, die wir immer wieder reproduzieren konnten, und die wir ausreichend oft beobachtet haben. Die gesetzmässigsten Erscheinungen haben wir dann schliesslich auch an *Katzen, Hunden* und *Affen* nachgeprüft. Für die letztgenannten Tiere mussten wir, was die Feldeigenströme betrifft, feststellen, dass dieselben auch bei diesen Tieren keinesfalls über der ganzen Hirnoberfläche synchron verlaufen. Die Feldeigenströme der einzelnen Felder weisen eindeutige qualitative und quantitative Unterschiede auf. Gleichzeitige mehrfache Ableitungen zeigten klar, dass *die Schwankungen* in ein und demselben Feld *selbst beim Affen* mehr oder weniger synchron und *in verschiedenen Feldern dyschron* verlaufen, wie wir an früherer Stelle (Kornmüller, 1933 *d, e*; Tönnies, 1933 *a*) bereits mitgeteilt haben. Typen von Feldeigenströmen beim Affen aufzustellen ist deswegen nicht so leicht, weil sich die einzelnen Frequenzen im Feldeigenstrombild meistens nicht so reinlich trennen wie beim Kaninchen, sondern häufiger Überlagerungen mehrerer Frequenzen zu beobachten sind, sodass erst durch genauere geometrische Analyse sich einwandfreie, zahlenmässig fassbare *FES*-Typen unterscheiden lassen. Vom Affenhirn gleichzeitig abgeleitete Feldeigenströme wurden in einer früheren Arbeit abgebildet (Kornmüller, 1933 *e*) und in 5-facher gleichzeitiger Registrierung von Tönnies auf der Jahresversammlung der Gesellschaft deutscher Nervenärzte, München 1934, gezeigt.

(b) Die Aktionsströme.

Auf *Augenbelichtung* ergab bei allen untersuchten Tieren stets die ausgeprägteste bioelektrische Antwort das architektonische Feld 17, die *Area striata* (Kornmüller, 1932–34). Dieses Feld ist auf den Hirnkarten der Fig. 5 (1–3) durch Schraffierung hervorgehoben. Konnten von Nachbarfeldern auch bioelektrische Belichtungseffekte abgeleitet werden, so hatten diese einen anderen Ablauf.

Beim Kaninchen besteht, wie Fig. 5 (1 *a*) darstellt, der Aktionsstrom der *Area striata* bei einer einzelnen Augenbelichtung in einer sehr raschen Anfangszacke gegen die elektro-positive Seite. Ihr folgen unmittelbar eine oder mehrere trägere Schwankungen, die weiter gegen die elektro-negative Seite hin reichen können. Bleibt der Aussenreiz unverändert fortbestehen, so kann das Bild der Feldeigen-

ströme voll oder abgeschwächt wieder in Erscheinung treten. Wird nun die Augenbelichtung plötzlich abgebrochen, so tritt meistens ein ähnlicher Effekt wie der Anfangseffekt auf. Der Endeffekt muss aber nicht immer die gleiche Ausprägung haben wie der Anfangseffekt. Erfolgen mehrere Reize rasch hintereinander, wie Fig. 5 (1 b) darstellt, so tritt das Aktionsstrombild meistens deutlicher in Erscheinung, besonders wenn es zu Superpositionen der Schwankungen zweier zeitlich benachbarter Effekte kommt. Aus dem Mitgeteilten ergibt sich, dass für das Zustande-
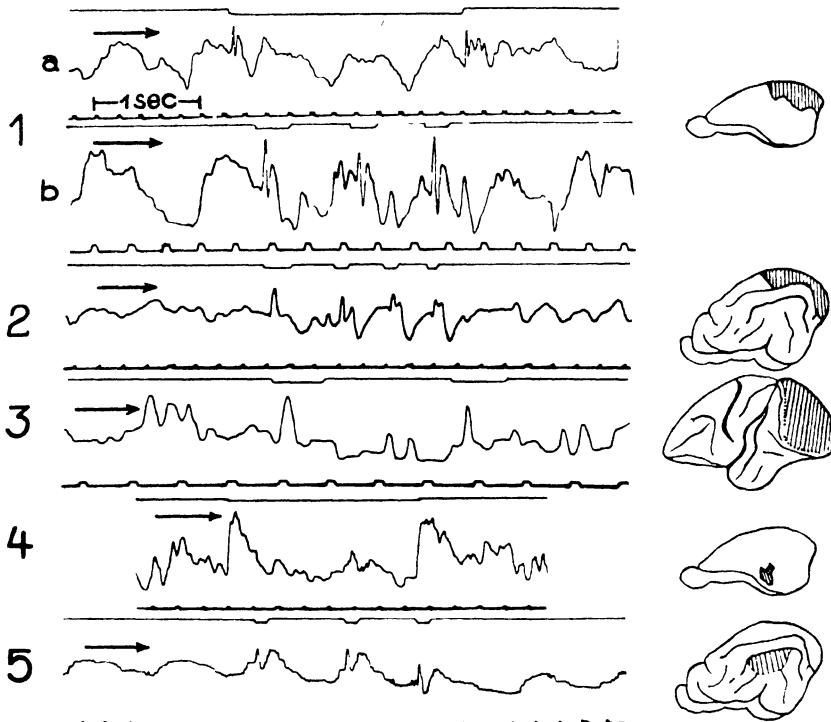
Fig. 5. Aktionsströme der Hirnrinde und deren Lokalisation. Oberhalb einer jeden Kurve die Reizmarkierung. Die Verlagerung dieser Linie gegen die Kurve hin gibt bei 1, 2 und 3 Augenbelichtung und bei 4 und 5 Schalleinwirkung an. 1 und 4 stammen vom Kaninchen, 2 und 5 von der Katze und 3 vom Affen. Hinter den Kurven sind die Hirnoberflächen dieser Tiere gezeichnet. Die Ableitefelder der vorangehenden Kurven sind schraffiert. Registriert mit dem Neurographen.

kommen der Aktionsströme die *Änderung* des Reizes allem Anscheine nach wesentlich ist.

Das beschriebene Normalbild des Aktionsstromes des Kaninchens wurde schematisch dargestellt. Schon an der gesunden Hirnrinde kann man finden, dass sich häufig zwei aufeinanderfolgende Aktionsströme deutlich unterscheiden. Von den vielen Faktoren, die diese Ungleichheit der Aktionsstrombilder bedingen mögen, können wir zwei mit Bestimmtheit angeben: (1) den Feldeigenstrom und (2) schädigende Einwirkungen auf die Grosshirnrinde.

Ohne Zweifel hängt die Gestaltung des Aktionsstromes davon ab, in welcher Phase des Feldeigenstromes die Erregung von der Peripherie die Hirnrinde antrifft. Ist z. B. der *FES* gerade in einer elektro-positiven Phase, so fällt die erste positive Schwankung des Aktionsstromes klein aus; sie ist gross, wenn die *FES*-Schwankung in diesem Zeitpunkt ganz auf der elektro-negativen Seite liegt. Viele der Ungleichheiten von aufeinanderfolgenden Aktionsströmen beruhen darauf, dass die Hirnrinde durch den tierexperimentellen Eingriff Schädigungen erfährt (siehe weiter unten!), die eine Labilität der Hirnrinde bedingen, sodass infolge der ständigen Zustandsänderung des Gehirns auch die Aktionsströme entsprechend grosse Abänderungen erfahren.

Lähmende Einwirkungen auf die Hirnrinde führen zunächst zum Kleinerwerden bezw. Verschwinden der ersten (raschen) Zacke des Anfangs- und Endeffekts. Bei Fortdauer der Einwirkung lässt die Hirnrinde keine bioelektrische Antwort auf einen Sinnesreiz mehr erkennen. Wird die Erregbarkeit der Hirnrinde gesteigert, so kommt es zu Amplitudenvergrösserung und zu einem Überwiegen sehr rascher Abläufe, die den Reiz auch um Vieles überdauern können (epileptiforme Reaktion). Diese leichte Beeinflussbarkeit des Aktionsstrombildes wurde von den meisten Autoren nicht berücksichtigt. Derartige Kurven fanden sogar noch eine Auswertung für rein physiologische Fragestellungen. Wir halten die Aktionsstrombilder für solche Fragen aus folgenden Gründen nicht besonders geeignet: Untersuchungen mit Tönnies[1] am *Kaninchen* (Kornmüller, 1933 *e*) haben ergeben, dass bei Anwendung von Reizen und Reizgefällen, wie sie in der Natur vorkommen, keinerlei Aktionsströme auftreten. Nur abnorm starke oder plötzliche Reize führten zu solchen. Dauerreize konstanter Intensität liessen bisher *meistens* keine Modifikationen der Feldeigenströme beobachten. Wie viele Figuren anderer Autoren zeigen, haben diese häufig ihre Aktionsstrombilder in einem Stadium gewonnen, wo keine Feldeigenströme mehr zu registrieren waren. Zu diesem Zeitpunkt, in dem sich die *Hirnrinde* in einem besonders labilen Zustand befindet, sind die *Aktionsstrombilder* zwar sehr deutlich, jedoch äusserst *variabel*. Es ist unseres Erachtens nicht angängig aus einem derartigen Material Schlussfolgerungen über die "Physiologie" der optischen Hirnrinde zu ziehen, und es sind auch in diesem Zusammenhange exakte Ausmessungen und Analysen, wie sie vorgenommen wurden nach unseren Erfahrungen von geringerer Überzeugungskraft. Siehe dazu Fig. 8 und Abschnitt III, 3 *d*!

Ohne Zweifel können die Aktionsströme aber als Indikatoren für die Lokalisation von Funktionen herangezogen werden. Wir haben deshalb ihre exakte *Lokalisation* in den Vordergrund gestellt.

Wie wir an früherer Stelle (Kornmüller, 1932, 1933) bereits an Hand von Hirnschnitten gezeigt haben, ist der oben beschriebene Aktionsstrom auf Augenbelichtung in seiner Ausdehnung auf der Hirnoberfläche ganz streng an das Feld 17, die *Area striata*, gebunden. Dieser Befund konnte an einer grossen Zahl von Gehirnen immer wieder bestätigt werden. Die Grenze der Ableitestelle des be-

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[1] Diese Untersuchungen werden fortgesetzt.

sagten Aktionsstromes ist streng koinzident mit der morphologischen Grenze der *Area striata* (Kornmüller, 1932, 1933, 1934).

Fig. 5 (2) zeigt die *Area striata* des Katzengehirns und daneben eine Registrierung von derselben. Die Reizmarkierung gibt Augenbelichtung an. Unmittelbar nach Beginn jeder Augenbelichtung erfolgt eine rasche an- und absteigende elektro-positive Schwankung, die nach einem kleinen abermaligen Anstieg von einer elektro-negativen gefolgt wird. Es sind nur Anfangseffekte und keine Endeffekte festzustellen. Der Feldeigenstrom, der vor und nach der rhythmischen Augenbelichtung zu sehen ist, zeigt verhältnismässig *träge* Schwankungen, wodurch allem Anscheine nach alle die Felder charakterisiert sind, die man als sensorische Felder zu bezeichnen pflegt.

Fig. 5 (3) stellt die *Area striata* eines Affenhirns dar und ausserdem eine Ableitung von demselben Feld des gleichen Tieres (*Cynomolgus*) ebenfalls bei Augenbelichtung. Wie die Zeitmarkierung (unten) erkennen lässt, war die Papiergeschwindigkeit bei dieser Registrierung etwas grösser als bei den anderen Kurven der Fig. 5. Die grossen Schwankungen, die zu Beginn der Augenbelichtung erfolgen, heben sich deutlich aus dem Bild der Feldeigenströme heraus.

Die Abgrenzung der bioelektrischen Belichtungseffekte beim Affen zeigt Fig. 1. Der Einstich (in der Mitte des Bildes) markiert die Grenze der Ableitestellen des in Fig. 5 (3) dargestellten Aktionsstromes auf Augenbelichtung, den nur die links davon gelegenen Gegenden ableiten liessen. Ausserdem wurden links und rechts von der durch den Einstich charakterisierten Stelle der Hirnoberfläche differente Feldeigenströme registriert. *Der Einstich ist streng koinzident mit der architektonischen Grenze* zwischen den *Areae* 17 (*Striata*) und 18. Eine Beschreibung der Fig. 1 befindet sich im Abschnitt I, 3.

Eine analoge scharfe Begrenzung nach architektonischen Feldern fand sich bei den Untersuchungen über die Lokalisation der Aktionsströme, die auf *Schalleinwirkung* zu registrieren sind. Beim Kaninchen waren diese an unsere *Area temporalis anterior*,[1] deren Lage aus Fig. 5 (4) zu ersehen ist, gebunden. Sie bestehen im wesentlichen in einem sehr raschen Ablauf gegen die elektro-positive Seite. Das Potential bleibt entweder über längere Zeit verlagert, oder aber es folgt ein allmähliches Zurückgehen desselben gegen die elektro-negative Seite (Fig. 5 (4)). Bei länger dauernder Schalleinwirkung wird diese Verlagerung gelegentlich durch vereinzelte Schwankungen von etwa 300 Sigmen Dauer unterbrochen, das sind trägere Schwankungen als der Feldeigenstrom zeigt. Bei den Tieren, die lediglich eine bleibende Potentialverlagerung zeigten, ging bei Reizende diese durch eine rasche Schwankung wieder zur Norm zurück. Bei den anderen Tieren wiederum, bei denen der elektro-positiven Schwankung eine sehr träge Verlagerung nach der elektro-negativen Seite folgte, ergab sich bei Reizende der gleiche Effekt wie zu Reizbeginn. Interessanterweise war der Endeffekt manchmal ausgeprägter als der Anfangseffekt.

Bei unseren vielen Erfahrungen konnten wir bis jetzt an keinem Tier bei

[1] Unsere *Area temporalis anterior* entspricht dem vorderen Teile der *Area temporalis I* nach M. Rose (1931).

akustischen Reizen raschere Schwankungen als die beschriebenen beobachten. Jedesmal, wenn wir sehr hohe Frequenzen registrierten, die sogar denen des dargebotenen Tones entsprachen, stellte sich bei genauer Prüfung heraus, dass es sich dabei nicht um *bio*elektrische Effekte, sondern um Störungen handelte.[1] Diese Feststellung betonen wir deswegen mit Nachdruck, weil bekanntlich Wever und Bray (1930) festzustellen glaubten, dass die Frequenz der Aktionsströme des *Nervus acusticus* der Frequenz des Tones, der das Ohr trifft, entspricht. Von anderen Autoren, z. B. Kreezer und Darge (1932), wurde dieser Auffassung bereits widersprochen.

Was die *Grosshirnrinde* betrifft konnten wir jedenfalls keinerlei Anhaltspunkte für einen Wever-Bray Effekt gewinnen.

Fig. 5 (5) stellt die Lokalisation und den Ablauf der Aktionsströme bei Schalleinwirkung an der Katze dar. Auf einen trägen Feldeigenstrom aufgebaut, besteht der Aktionsstrom aus einer raschen elektro-positiven Schwankung, die von einer trägen gefolgt wird. Bei kürzerer Schalleinwirkung lässt sich nur ein Anfangseffekt feststellen. Das Ableitefeld liegt in der Meynertschen Katzenanastomose und fällt räumlich mit der *Area* 52 nach Brodmann (1909) zusammen. Diese Gegend hatte C. Vogt (1900) als frühmarkreif gefunden und mutmasslich als Hörzentrum angesprochen. Seit *Munk* wird dagegen von vielen Autoren (Horsley und Schafer, Larionow, Rothmann u.a.—Lit. bei Graham Brown (1927)) der *Schläfenlappen* als Hörsphäre angegeben und viel diskutiert. Auf Grund unserer Befunde können wir uns aber dieser Auffassung nicht anschliessen.

An den bisher untersuchten Tieren (Katzen und Kaninchen) konnten wir in einer mit Tönnies (Kornmüller, 1933 *a*) angestellten Versuchsreihe keinerlei skalenförmige Anordnung der Ableitestellen auf verschiedene Tonhöhen finden. Ein jeder Ton liess bioelektrische Effekte von dem ganzen in Frage kommenden Areal ableiten. Dieser Befund spricht nicht für die verbreitete Ansicht einer Lokalisation nach Tonhöhen in der Hörsphäre (Munk, 1890; Larionow, 1899; Bechterew u.a.), welcher Ansicht von einigen Autoren (Börnstein, 1930, u.a.) bereits widersprochen wurde.

Gerard, Marshall und Saul (1933) berichten, dass bei der Katze die Hirnrinde keine bioelektrische Beantwortung akustischer Reize bei ihren Untersuchungen erkennen liess. Unsere oben ausgeführten positiven Befunde stehen diesen gegenüber. Beim Affen wurden von diesen Autoren akustische Aktionsströme von der Oberfläche des ganzen Temporallappens abgeleitet. Letzteres ist uns nie gelungen trotz wiederholter Versuche. Lediglich von der Area 52, einem latogranulären Feld der Sylvischen Furche, konnten wir am *Cynomolgus* Aktionsströme erhalten. Vergleichend architektonisch entspricht dieses Feld dem oben erwähnten "akustischen" Felde des Kaninchens und der Katze. Grosses Interesse dürften die von den genannten Autoren vorgenommenen subkortikalen Ableitungen beanspruchen.

Nach den eindeutigen bisher mitgeteilten Befunden kann es nicht schwer fallen auch bei anderen Arten von Sinnesreizen Aktionsströme der Grosshirnrinde abzuleiten und diese exakt zu lokalisieren. Wesentlich bei solchen Experimenten ist

[1] Dies gilt auch für die Abb. 6 der Arbeit von Fischer (1932).

die Möglichkeit, scharf begrenzte Reize zu setzen. In einer langen, unveröffentlichten Versuchsreihe mit M. Vogt haben wir uns bemüht, Aktionsströme bei Hautreizen und passiven Bewegungen zu registrieren. Trotz der vielen Experimente, die hauptsächlich an Kaninchen und Katzen ausgeführt wurden, konnten wir bei hautsensiblen Reizen keinerlei Aktionsströme von der Hirnrinde ableiten. Wir sind deshalb zu der Annahme gekommen, dass bei den genannten Tieren eine kortikale Vertretung dieser Sinne möglicherweise nur sehr spärlich oder gar nicht vorhanden ist. Nur scheinbar kann man bei derartigen Experimenten Effekte bekommen, doch mussten sich diese bei genauerer Prüfung immer als Störungen, wie sie durch Berührung des Tieres hervorgerufen werden, erweisen. Ein Kunstprodukt ist auch die aus gemeinsamen Untersuchungen mit dem Verfasser stammende Kurve der Abb. 8 in der Arbeit von Fischer (1932), die ausserdem ein vereinzelter Befund geblieben ist.

Da die Aktionsströme wichtige Indikatoren für die Lokalisation von Sinnesfunktionen darstellen, halten wir es für wertvoll, in nächster Zeit an die Ableitung von Aktionsströmen bei Geschmacks- und Geruchsreizen heranzugehen, da besonders über die Lokalisation dieser Funktionen noch grosse Unklarheit besteht. Die viel diskutierte und trotzdem bis vor kurzem noch völlig unklare kortikale

Fig. 6. Beruhigung der Feldeigenstrome der *Area occipitalis* (*Occ* der Fig. 2) auf rhythmische Schalleinwirkung. Registriert mit dem Neurographen.

Lokalisation des *Vestibularis* erfuhr durch Spiegel (1933, 1934) eine Bearbeitung. Der genannte Autor versucht neuerdings die mittels der *Strychnin*methode (siehe weiter unten!) gewonnenen Ergebnisse durch Ableitung des "Encephalogramm" zu erhärten. Er lokalisiert den *Vestibularis* bei der Katze in ein Gebiet, das vom vorderen Abschnitt des *Gyrus suprasylvii* bis in die Gegend der Meynertschen Anastomose reicht.

Auf einen peripheren Sinnesreiz hin zeigt also je nach der Art des Reizes ein bestimmtes streng umschriebenes Areal der Grosshirnrinde einen Aktionsstrom. Der *überwiegend grössere Teil der Grosshirnrinde liess keinerlei bioelektrische Beantwortung des Aussenreizes erkennen.* Dies war selbst bei Einwirkung hypermaximaler Reize in einer grossen Reihe von Experimenten immer wieder festzustellen. Nur vereinzelt und dann auch nur über bestimmten Feldern waren *gelegentlich Beruhigungen* der Feldeigenströme bei der Einwirkung von Sinnesreizen festzustellen, wie dies Fig. 6 zeigt.

Da die Kurve von Fig. 6 eine Ausnahme bildet gegenüber dem viel häufigeren Befunde, dass die Nachbarfelder unbeeinflusst bleiben, kann also nicht die Rede davon sein, dass im Zentralnervensystem jeder Vorgang ein Vorgang des Gesamtsystems sei, wie dies von manchen Autoren vertreten wird. Wir kommen auf Grund unserer Ergebnisse weiter unten auf das umstrittene Lokalisations- bezw. Ganzheitsproblem des Zentralnervensystems zu sprechen.

(2) Schlussfolgerungen und Prinzipielles zum Lokalisationsproblem.

Aus dem Bisherigen können wir folgende *allgemeinere Schlussfolgerungen* ziehen:

(1) *Die normale Grosshirnrinde lässt sich auf Grund ihrer bioelektrischen Erscheinungen regional differenzieren.*

(2) *Die Unterschiede der einzelnen bioelektrischen Areae beziehen sich auf die Form und Grösse ihrer Stromschwankungen. Über ein und demselben bioelektrischen Feld sind die Stromschwankungen weitgehend synchron, über verschiedenen Feldern dagegen dyschron.*

(3) *In ihrem bioelektrischen Verhalten einheitliche Hirnrindenareale decken sich stets exakt mit architektonischen Feldern.*

(4) *An einer weitgehenden strengen Lokalisation der bioelektrischen Erscheinungen der Grosshirnrinde besteht demnach kein Zweifel.*

Bevor wir auf Grund dieser feststehenden und an einem grossen Material erhärteten Tatsachen zum *Problem der Lokalisation auf der Grosshirnrinde* Stellung nehmen, bringen wir einen kurzen Überblick über die Entwicklung dieses Problems. Diese Zusammenstellung will nicht über den Rahmen dieser Arbeit hinausgehen und erhebt darum keinen Anspruch auf Vollständigkeit.

Durch F. J. Galls *Phrenologie* wurde die Annahme angeregt, dass die einzelnen Funktionen eine gesonderte Lokalisation im Gehirn haben. Das gleiche wurde durch den aufsehenerregenden Befund an zwei Gehirnen hervorgerufen, den Broca (1877) im Jahre 1861 mitteilte. Dieser bestand darin, dass an den beiden Gehirnen Defekte in der zweiten und dritten Stirnwindung zum Unvermögen zu sprechen (motorische Aphasie) geführt hatten, womit Broca den Nachweis zu erbringen suchte, dass eine bestimmte psychische Funktion an die Intaktheit eines begrenzten Hirnteiles gebunden ist. Eine grosse Zahl namhafter Autoren versuchte mit Hilfe verschiedener Methoden eine Reihe von Funktionen im Gehirn zu lokalisieren (Fritsch; Hitzig; Ferrier, 1879, 1892; Luciani und Mitarbeiter, 1886; Horsley und Schafer; Monakow; Bianchi; Tonnini; Christiani; Munk, 1890; Flechsig; und viele andere). Diese Untersuchungen haben für die Entwicklung des Lokalisationsproblems grosse Bedeutung. Sie sind auch nicht ohne Nutzen für die praktische Medizin geblieben. Dies bleibt bestehen, wenn auch zu einer späteren Zeit verschiedene Mängel an diesen Untersuchungen aufgedeckt wurden. Der Hauptmangel ist wohl der, dass diese Autoren in fest abgegrenzte Hirnregionen—die architektonischen Felder waren damals so gut wie unbekannt—Einheiten lokalisiert haben, die psychologische Begriffe von oft recht komplexer Natur darstellen, wie zum Beispiel die "Intelligenz", das "Wortgedächtnis" und ähnliches mehr. Dabei blieb die prinzipielle Frage unbeantwortet, ob es überhaupt angängig ist, in kleine Hirngebiete komplexe psychologische Einheiten zu lokalisieren.

Es ist darum nicht sehr verwunderlich, dass schon frühzeitig Stimmen laut wurden gegen die Lokalisation komplexer psychischer Funktionen (Wundt; Brodmann, 1909; u. a.). Auch von seiten der Kliniker kamen solche Bedenken, vorerst durch Goldstein (1927), der eine Verfeinerung der psychologischen Analyse, die zu

psychischen Elementen führen sollte, propagierte. Der Genannte hat den Wert anatomischer Tatsachen für eine Lehre der Funktionen des Nervensystems eindeutig in Abrede gestellt. Er ging dabei vor allem von Theorien und Tatsachen der Psychologie aus und zog dazu Befunde heran, wie sie durch Bethe (1903) an niederen Tieren gemacht wurden und zu einer Lehre von der "Plastizität des Nervensystems" geführt hatten. Ähnliche Schlussfolgerungen wie Goldstein zieht Lashley (1929–32) aus seinen Untersuchungen. Im Gegensatz zu den genannten Autoren haben wir keinen Zweifel daran, dass sich in wenigen Jahren *die Lehre durchsetzen wird von der strengen Gebundenheit der spezifischen Funktionen an spezifische Strukturen des Gehirns.* Alle unsere Befunde sprechen mit Entschiedenheit dafür.

Schon seit jeher stehen sich zwei Lager gegenüber, die Vertreter einer *strengen* Lokalisation der Funktionen auf der Grosshirnrinde und die bereits teilweise angeführten Gegner einer solchen. Extreme Lehren gipfeln sogar in dem Satz, dass im Zentralnervensystem "jeder Vorgang ein Vorgang des Gesamtsystems sei" (v. Weizsäcker).[1] Da das *ZNS* ein Syncitium darstelle, müsste jeder Reiz das *ganze* System treffen. Dass dies aber in Wirklichkeit *nicht* der Fall ist, beweisen eindringlichst unsere Befunde. Selbst auf hypermaximale Sinnesreize zeigte der überwiegende Teil der Grosshirnrinde keinerlei bioelektrische Antwort. Die vielen unterschiedlichen Typen von Feldeigenströmen und ihr Dyschronismus, wie gleichzeitige mehrfache Ableitungen feststellen liessen, *entkräften die angeführte "Ganzheitsauffassung" vom ZNS.* Wir sind aber auf Grund unserer Tatsachen keinesfalls der Auffassung, dass jedes Hirnrindenfeld ganz selbständig ist. Es bestehen viele gerichtete Beziehungen bestimmter, aber keinesfalls aller Felder untereinander, was schon morphologische Tatsachen (Faserverbindungen) erwarten lassen. Diese lassen sich aus teilweisen Synchronismen gewisser Stromschwankungen feststellen, wie wir weiter oben dargelegt haben.

Als Forderungen für lokalisatorische Untersuchungen stellen wir folgende Bedingungen auf:

(1) Unbekümmert um eine Lokalisation von Funktionen sind vorerst mit allen zur Verfügung stehenden exakten Methoden der Physiologie, Physik und Chemie weitere Differenzen in der Grosshirnrinde und überhaupt im Zentralnervensystem aufzuzeigen, was gewiss weitgehend gelingen wird, da die weitgehende Differenzierung des Baues solche erwarten lässt und die Ergebnisse aller daraufhin angestellten Untersuchungen durchaus in diesem Sinne sprechen. Von diesem festen Boden aus könnte man in vielen Jahren mit mehr Erfolg an die Lokalisation von Funktionen herangehen.

(2) Reinlichste Trennung zwischen den tatsächlichen Befunden und deren Interpretation im Sinne einer Lokalisation von Funktionen. Darauf wurde bis jetzt zu wenig geachtet. Dies ist aber in allen Fällen sehr nötig, vor allem dann, wenn man die Lokalisation psychischer Funktionen zu bearbeiten versucht. Das ist besonders angezeigt, wenn es sich, wie immer bei exakten Untersuchungen, um Experimente an *Tieren* handelt, deren "Psyche" für uns fast völlig unzugänglich ist.

[1] Zitiert nach Wachholder (1932).

Da das Lokalisationsproblem in das Gebiet der Biologie gehört, ist es meines Erachtens unangängig, zum Ausgangspunkt Theorien oder Prinzipien der Psychologie zu machen. Damit sei nicht im geringsten der grosse Wert, den die Psychologie an sich hat, angetastet.

(3) Die Morphologie und im besonderen die Architektonik ist die sicherste Basis für alle lokalisatorischen Untersuchungen, denn sie hat uns, was bereits in der Einleitung zu dieser Arbeit ausgeführt worden ist, eine grosse Fülle eindeutiger Strukturdifferenzen aufgezeigt und so eine weitgehende Gliederung der Hirnrinde in scharf voneinander abgegrenzte architektonische Felder ermöglicht. Wenn man auch über die Funktionen der meisten dieser Felder zur Zeit so gut wie nichts aussagen kann, so ist wohl die folgende Schlussfolgerung evident: *Die eindeutigen Unterschiede im Bau, die die einzelnen architektonischen Felder charakterisieren, sind Indikatoren dafür, dass die einzelnen Felder nicht gleiche, sondern verschiedene physiologische bezw. funktionelle Bedeutung haben.* Es ist anzunehmen, dass ungleich gebaute Felder eine verschiedene Funktion besitzen. Dass eine der von der Psychologie unterschiedenen Funktionen an *ein* solches Feld allein gebunden sein soll, glauben wir nicht. Derartig komplexe Funktionen werden sich wohl auf Grundlage eines sehr komplizierten Mechanismus, der gleichzeitig viele Felder und subkortikale Kerne beansprucht, ergeben. Dabei aber wird der Funktionsanteil verschieden gebauter *Areae* bezw. Kerne ein unterschiedlicher und spezifischer sein.

(3) Zur Physiologie des Zentralnervensystems.

Von allen Organen unterscheidet sich das Gehirn durch die ungleich grössere Zahl von Baueinheiten mannigfaltigster Gestaltung und Verknüpfung. Darum können auch alle Untersuchungen, bei denen das Gehirn als eine Einheit betrachtet wird, nur sehr grobe Befunde liefern. Hierin liegt der Grund, weshalb wir uns zuerst eingehend mit der Frage der *Lokalisation* der bioelektrischen Erscheinungen beschäftigt haben. Auf dieser Grundlage seien im folgenden einige Probleme der *Physiologie* des *ZNS* behandelt. In den Abschnitten *a–c* halten wir uns teilweise an eine gemeinschaftliche Untersuchung mit Tönnies.

(a) *Quantitatives über den elektrischen Energiewechsel der Grosshirnrinde.*

Um den elektrischen Energiewechsel messend zu erfassen, kann man an den Kurven die Millivolt-Produktionen pro Zeiteinheit (mV/sec) ausmessen, wie wir es getan haben. Ohne Zweifel ist der elektrische Energiewechsel der Grosshirnrinde und überhaupt der Grisea normalerweise grösser als der des Marklagers und des peripheren Nervensystems, was sich schon aus der Tatsache der ständig vorhandenen Ströme der Grisea ergibt. Dass der elektrische Energiewechsel in seinem zeitlichen Ablauf nach Feldern eindeutig verschieden ist, braucht hier nicht näher ausgeführt zu werden. Dies ergibt sich schon aus Fig. 2. In quantitativer Hinsicht

finden wir für die *normale* Grosshirnrinde des Kaninchens über den einzelnen Ab!eite-*Punkten* Spannungsproduktionen in der Grössenordnung von ungefähr 3 bis 8 Millivolt Spannungswechsel pro Sekunde. Die mittlere Amplitude der grösseren Spannungsschwankungen des gesunden Kaninchens liegt zwischen etwa 0·7 und 1·5 Millivolt. Unter Bedingungen, die eine Verschlechterung gegen den normalen Zustand bedeuten, kann die Amplitude bis auf 0·3 Millivolt und auch noch weiter herabsinken. Diese Zahlen wurden wie auch die folgenden an einem statistisch ausreichenden Material gewonnen, stellen aber trotzdem wegen der individuellen Verschiedenheiten nur ganz grobe Werte dar. Durch abnorme Reize (mechanische und elektrische Reizung der Hirnrinde, Hirnkrampfgifte und anderes mehr) können grosse Steigerungen des elektrischen Energiewechsels hervorgerufen werden. Allermeist sind derartige Phasen gefolgt von Stadien, die keine oder nur verminderte Spannungsproduktionen aufweisen. In verschiedenen Fällen konnten wir durch Ausmessung *Steigerungen* der mV/sec *um das* 20-*fache* im Verhältnis zu den normalen Feldeigenströmen feststellen. Es konnten Spannungsschwankungen von 7 Millivolt und gelegentlich auch noch mehr gemessen werden, also in Bezug auf die Amplitude mehr als 7-fache Werte als beim gesunden Gehirn; während die raschesten Frequenzen, die wir am *gesunden* Kaninchenhirn feststellen konnten, etwa 35 Hertz bei sehr kleiner Amplitude betrugen, haben wir bei Krampfströmen Frequenzen von mehr als 60 Hertz registriert. Diese Tatsache der Steigerung des elektrischen Energiewechsels zeigt sich analog den Feststellungen von Winterstein (1929), welcher z. B. bei elektrischer Reizung des Zentralnervensystems ein starkes Ansteigen des Gaswechsels (O_2-Verbrauch, CO_2-Bildung) fand, der bei normaler Tätigkeit des Gehirns nicht festzustellen ist. Winterstein kam auf Grund seiner Untersuchungen dazu, am Zentralnervensystem einen Ruhe- und Tätigkeits-stoffwechsel zu unterscheiden; von letzterem trennt er aber einen *Reizstoffwechsel* ab, der bei elektrischer Reizung mit einem *Ansteigen des Gaswechsels* zu beobachten ist. Allerdings sind uns mehr Ursachen für das Zustandekommen von Krampf-strömen bekannt, als Winterstein für das Auftreten eines "Reizstoffwechsels" angegeben hat. Hier seien nur genannt mechanische Reizung und die verschie-densten chemischen Wirkungen, die entweder lokal oder auf dem Blutwege das Gehirn trafen. Siehe weiter unten!

Wintersteins Auffassung ist allerdings nicht unwidersprochen geblieben. Gerard und Hartline (1934) u.a. haben sich dagegen geäussert.

(b) *Zum Erregungsablauf und zum Stoffwechsel der Grosshirnrinde.*

Für das periphere Nervensystem wird allgemein der Satz anerkannt, dass die "Stärke, Dauer und Häufigkeit von Aktionsströmen" "uns ein getreues Bild von der Stärke, Dauer und Häufigkeit der über den Nerven ablaufenden Erregungen" gibt. (Zitiert nach Wachholder, 1932.)

Wir glauben zu der Annahme berechtigt zu sein, dass auch für das Zentral-nervensystem ein enger Zusammenhang zwischen bioelektrischen Abläufen und seiner Tätigkeit besteht. Dass diese Beziehung, was die Zeit betrifft, sehr eng und eindeutig ist, darf ohne weiteres angenommen werden. Ob sie in quantitativer und

qualitativer Hinsicht in gleicher Weise besteht wie für das periphere Nervensystem, wird weiter unten ausgeführt. (Siehe "Arbeitshypothese"!)

(1) Wie die bioelektrischen Kurven zeigen, ist der *Tätigkeitsablauf auf der Grosshirnrinde* nicht überall der gleiche, sondern *feldmässig verschieden*. Siehe Fig. 2! Aus diesen Kurven ergibt sich, dass die untersuchten Felder des normalen Gehirns ständig tätig sind. Diese *ständige Tätigkeit der normalen Grosshirnrinde* bleibt fortbestehen, selbst wenn alle Aussenreize so gut wie möglich ausgeschaltet und keinerlei effektorische Leistungen des Individuums zu beobachten sind. Die *Area striata*, das Feld, welches auf Lichtreize Aktionsströme produziert, zeigt beispielsweise Feldeigenströme auch dann, wenn sich das Versuchstier in einem ganz verdunkelten Raume in völliger motorischer Ruhe befindet. Diese Erkenntnis erscheint uns aus mancherlei Gründen nicht ohne Bedeutung. Sie führt uns eine *weitgehende Autonomie der Hirnrinde* vor Augen. Wird durch einen peripheren Sinnesreiz eine Erregungswelle hervorgerufen, so trifft diese die Hirnrinde also nicht in Ruhe, sondern mit ständigen Erregungsabläufen an. Den letzteren möchten wir darum einen wesentlichen Anteil am Zustandekommen der funktionellen Leistungen geben. Das Hirngeschehen kann deshalb nicht einfach durch eine zentrale Projektion der Sinnesorgane und des übrigen peripheren Organismus verstanden werden. Es ist anzunehmen, dass das *Wechselspiel zwischen zentralen Erregungsabläufen und den von der Peripherie kommenden Erregungen* für das Zustandekommen zentralnervöser Leistungen wesentlich ist.[1] Auch das Entstehen der *Willkürinnervation* kann man sich auf dem Boden ständig vorhandener Erregungen leichter vorstellen, als wenn man annehmen müsste, dass in einem völlig ruhigen Hirngebiet plötzlich Erregungen sozusagen aus dem Nichts entstehen.

(2) Wie Fig. 2 ausserdem zeigt, ist *der Erregungsablauf in vielen Feldern der Grosshirnrinde* meistens *ein periodischer*. Die *Periodizität* ist aber *von Feld zu Feld verschieden und für jede Area eine spezifische*. Dass dem elektrischen Energiewechsel entsprechend der *Ablauf der Stoffwechselvorgänge* zumindest in zeitlicher und quantitativer Hinsicht ebenfalls nicht einheitlich, sondern feldmässig verschieden ist, scheint verständlich. Felder, deren bioelektrische Spannungsproduktionen kleiner und spärlicher sind, dürften wohl auch einen geringeren Stoffwechsel haben.

Abänderungen des normalen Gaswechsels haben uns einen grossen Einfluss auf das bioelektrische Bild gezeigt. Früher haben wir häufig an kuraresierten Tieren gearbeitet. Die dabei notwendige künstliche Ventilation wurde meistens während der Registrierungen unterbrochen. Dabei zeigte sich, dass sehr bald die Amplituden der Feldeigenströme abnahmen. Bei länger dauerndem Aussetzen der Atmung verschwanden schliesslich auch die Aktionsströme. Bei Asphyxie werden die Stromschwankungen fast augenblicklich abnorm, und in weniger als 1 Minute kann nach vorherigen Krampfstromabläufen die elektrische Spannungsproduktion völlig aufhören, was wohl ein Erlöschen der Tätigkeit der betreffenden Ableitestelle anzeigt. Bei frühzeitiger, ausreichender Ventilation ist eine Restitution der elek-

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[1] Auf Anregung von O. Vogt sind Untersuchungen im Gange, bei denen der Anteil der Peripherie am Zustandekommen der Eigenströme festgestellt werden soll.

trischen Stromschwankungen und somit auch der Tätigkeit möglich. Durch Hyperventilation eines Versuchstieres konnten immer wieder Krampfströme ausgelöst werden. Während Äthernarkosen wurde im Augenblick akuter Atemlähmung nahezu gleichzeitiges Aussetzen der Spannungsproduktion beobachtet, obgleich meistens noch länger als eine halbe Stunde hindurch eine Herzaktion mittels des *EKG* festzustellen war. Diese und andere Ergebnisse deuten auf eine *innige Beziehung zwischen Gasstoffwechsel und bioelektrischen Erscheinungen, bezw. der Tätigkeit der Grosshirnrinde*, hin. Dabei müssen wir aber ausdrücklich feststellen, dass es keinerlei Beziehungen zwischen dem Rhythmus der Atmung, des Herzens und der Hirnpulsation einerseits und den bioelektrischen Wellen andererseits gibt. Darauf haben wir wiederholt unsere besondere Aufmerksamkeit gerichtet.

Auf Grund anderer Methoden ist bekannt, dass das Zentralnervensystem von allen Organen des Körpers den weitaus grössten oxydativen Gaswechsel hat. (Lit. bei Winterstein, 1929 und Wachholder, 1932.) Pro Gewicht- und Zeiteinheit ist der Gaswechsel des Gehirns, und zwar dessen Grund- oder Ruhegaswechsel, etwa 20mal so gross wie der Gaswechsel des ruhenden Skelettmuskels und der der grauen Substanz des Gehirns wahrscheinlich sogar 10–20mal so gross wie im peripheren Nervensystem. Andererseits aber geht aus Versuchen über den Gasstoffwechsel des Gehirns bei dessen "Tätigkeit" bis jetzt nicht hervor, dass z. B. bei geistiger Tätigkeit eine eindeutige Steigerung des Gaswechsels eintritt (Rubner, Cramer, Speck, Benedict und Carpenter; Lit. bei Wachholder, 1932). Auch die von Alexander (1912) durchgeführten Untersuchungen über den Einfluss intensiver Beleuchtung der Augen auf den Gaswechsel des Gehirns erbringen nach Wachholder nicht den einwandfreien Beweis einer Steigerung des Gaswechsels unter den genannten Bedingungen.

Dem grossen Gaswechsel des Gehirns können wir die Tatsache der ständig vorhandenen Ströme an die Seite stellen. Diese elektrischen Spannungsschwankungen, die das periphere Nervensystem nicht aufweist, zeigen uns einen vielfach grösseren Stoffwechsel der Grosshirnrinde an. Dass unter diesen Umständen auch ein entsprechender Mehrbedarf an Sauerstoff vorhanden ist, scheint durchaus verständlich. Dass aber keine besondere Steigerung des an und für sich grossen Gaswechsels des Gehirns bei dessen "Tätigkeit" auftritt, ist auf den ersten Blick erstaunlich. Unsere Untersuchungen aber ergeben, dass bei besonderen Erregungen des gesunden Grosshirns keine erhebliche Steigerung des elektrischen Energiewechsels eintritt. Dafür können wir folgendes anführen:

(1) Die Amplituden, also die Spannungswerte der Aktionsströme sind durchschnittlich kaum grösser als die der Feldeigenströme.

(2) Die auf einen bestimmten Sinnesreiz mit einem Aktionsstrom antwortenden Rindenfelder sind an Ausdehnung verschwindend klein im Verhältnis zur Fläche der übrigen Hirnrinde.

(3) Während der Reizeinwirkung konnte manchmal eine deutliche Beruhigung der Feldeigenströme vereinzelter anderer Felder beobachtet werden, was wohl auf eine verminderte Tätigkeit derselben schliessen lässt.

In quantitativer Hinsicht finden wir also für den elektrischen Energiewechsel

keine fassbaren Unterschiede zwischen einem "Grund"- oder "Ruhe"energie-
wechsel und einem "Tätigkeits"energiewechsel, wenn man das Gehirn in seiner
Gesamtheit betrachet. Wir möchten darum die Frage aufwerfen, ob es überhaupt
angängig ist, am normalen Zentralnervensystem einen Grund- oder Ruhestoff-
wechsel und einen Tätigkeitsstoffwechsel zu unterscheiden. Wir neigen dazu, diese
Frage zu verneinen, da uns die Tatsache ständig vorhandener Ströme gezeigt hat,
dass man von einer "Ruhe" des normalen Gehirns wohl nicht sprechen kann. Es
ist sehr wahrscheinlich, dass die einzelnen Felder unter verschiedenen Bedingungen
Unterschiede im Gaswechsel und überhaupt im Stoffwechsel aufweisen. Betrachtet
man aber, wie dies bei den bisherigen Untersuchungen meistens der Fall war und
aus methodischen Gründen sein musste, das Gehirn als eine Einheit, so ist unseres
Erachtens der Befund richtig, dass bei der Tätigkeit des Gehirns keine greifbare
Steigerung des an und für sich grossen Gaswechsels messbar ist.

Den von Holmes (1930) gemachten Befund, dass die weisse Substanz des
Gehirns einen geringeren Sauerstoffverbrauch hat als die graue, glauben wir durch
unsere Feststellung einer geringeren Spannungsproduktion des Markes gegenüber
der Rinde (Kornmüller, 1932 a) stützen zu können.

(c) Die spezifische Transformation der Erregungen im Zentralnervensystem.

Vornehmlich auf Grund von Studien über Reflexe hat sich ergeben, dass das
Zentralnervensystem auf Einzelreize durch langdauernde rhythmische Entladungen
antworten kann. Daraus hat sich die Auffassung gebildet, dass die Erregungen im
Zentralnervensystem eine weitgehende Umformung erfahren. Vom peripheren
Nervensystem dagegen nimmt man an, dass es die Erregungen unter normalen
Bedingungen unverändert weiterleitet.

Die bioelektrische Methode gestattete nun, von einer ganz anderen Seite her
diese Frage zu prüfen. Zu diesem Zweck wurden Ableitungen vom Optikus und
der *Area striata*, der kortikalen Endstation dieses Nerven, vorgenommen.[1] Das
Ergebnis zeigt Fig. 7. Die obere Kurve stellt die Aktionsströme des Optikus auf
Augenbelichtung dar. Unten sieht man die Aktionsströme der *Area striata* unter
den gleichen Bedingungen. Diese beiden Registrierungen wurden unter mehr-
facher Wiederholung abwechselnd vom Optikus und Rinde am gleichen Ver-
suchstier vorgenommen. Die grosse Differenz sowohl in der Form der Abläufe als
auch in der Grösse der Spannungsproduktionen kommt dabei deutlich zum Aus-
druck. Besonders bemerkenswert ist die Tatsache, dass die Hirnrinde einen ein-
zelnen Aussenreiz mit vielen rhythmischen Schwankungen beantwortet. Es müssen
also auch die Erregungsabläufe an der Peripherie und im zentralen Organ wesentlich
voneinander verschieden sein, d. h. es hat eine Transformation der Erregungen
stattgefunden. Diese Umformung erfolgt wohl in den Grisea. Die markhaltigen
Fasern leiten nur einen Teil der dort entstehenden Erregungen in einfacher Form

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[1] Von den *Corpora quadrigemina* und dem *Thalamus* wurden auch bereits lokalisierte Aktions-
strome auf Augenbelichtung von uns registriert.

weiter, die wiederum bei der Ankunft in neuen Ganglienzellen Erregungen gestei-
gerter Intensität explosionsartig hervorrufen.

Unserer Meinung nach bestehen zwischen dem peripheren und dem zentralen
Nervensystem nicht nur quantitative Unterschiede, wie dies häufig behauptet
wurde. Den Unterschied in der Physiologie dieser beiden Systeme halten wir für
einen prinzipiellen. Die Physiologie des Zentralnervensystems ist vorwiegend durch
die Tätigkeit der Ganglienzellen und die des peripheren Nervensystems durch die
der Nervenfasern bestimmt.
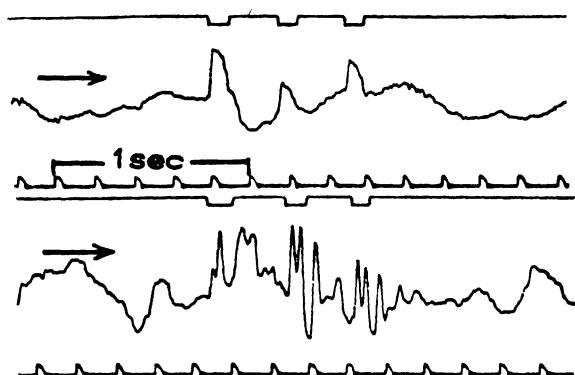
Fig. 7. Oben, Aktionsstrome des *Nervus opticus* und unten, die der *Area striata* desselben Kaninchens
bei rhythmischer Augenbelichtung. Registriert mit dem Neurographen.

*(d) Die bioelektrischen Erscheinungen bei abnormen Zuständen der Grosshirnrinde.
(Einwirkung von exogenen Schädlichkeiten, Hirngiften und Narkose. Kontrolle
hirnphysiologischer Methoden.)*

Unserer Registriermethode mit dem Neurographen haben wir es vor allem zu
verdanken, dass wir wohl das grösste Kurvenmaterial besitzen. Auf Grund dieses
ungeheueren Materials konnten wir die normalen bioelektrischen Erscheinungen
beschreiben, und es liess sich auch ein Überblick über die *Modifikationen* des
normalen bioelektrischen Bildes gewinnen.

Das Studium der *Modifikationen des bioelektrischen Normalbildes* verspricht
schon jetzt *vielfältige Förderung unserer Kenntnisse über die Pathologie des Gehirns
und des übrigen Zentralnervensystems.*

Grob betrachtet lassen sich von den oben beschriebenen bioelektrischen
Normalbildern ausgehend (1) Verminderungen und (2) Steigerungen der bio-
elektrischen Spannungsproduktion unterscheiden. Sind die oben beschriebenen
Bilder der Ausdruck des normalen Tätigkeitsablaufes in den Grosshirnrinden-
feldern, so zeigt die Verminderung wohl eine *Lähmung* und die Steigerung eine
Reizung der jeweiligen Ableitestelle an. Darüber hinaus gibt uns die bioelektrische
Methode neben vielem anderen noch genaue Auskunft über den *zeitlichen Ablauf*
der Zustandsänderung eines Hirnteiles.

Als *Ursachen* für solche Modifikationen kommen die verschiedensten exogenen
und endogenen Beeinflussungen des Gehirns in Frage. (Siehe Kornmüller, 1933 *a*

und *e*!) Es seien hier nur einige Beispiele angeführt. *Mechanische* und *thermische* Einwirkungen auf die Hirnoberfläche ergaben starke Zunahme der Spannungs-produktionen im Sinne von Krampfströmen und in deren Gefolge entweder Aufhören oder Verminderung der Spannungsproduktion. *Auskühlen* der Hirn-oberfläche führte meistens nur zu Verminderung und Aussetzen der Spannungs-produktionen. Fig. 8 zeigt den Einfluss einer Zimmertemperatur von etwa 17° C. auf das blossliegende Gehirn. In fortlaufender Registrierung zeigte sich, dass das Normalbild (*a*) eines Feldes (*Pc* + *Par*) schon nach etwa 3 Minuten eine Ver-minderung der Amplituden (*b*) aufwies. Nach etwa 5 Minuten vom Beginn dieser
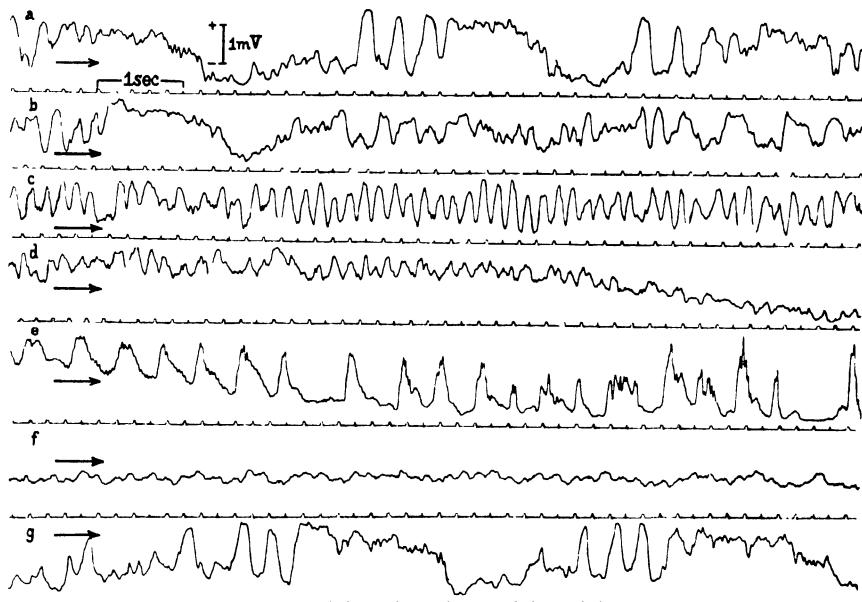
Fig. 8. *a–f*, Einwirkung von Kälte (etwa 17° C.) auf einen Feldeigenstrom (*Pc* + *Par*). *g*, Restitution des normalen Typus nach Wärmung der Hirnoberfläche mit körperwarmer physiologischer NaCl-Lösung. Registriert mit dem Neurographen.

Registrierung sind, wie Streifen *c* zeigt, die kleinen raschen Abläufe nahezu verschwunden. In nahezu ununterbrochener Reihenfolge tritt eine *neue Frequenz* von etwa 5 Hertz auf, die sich wohl aus den 3 Hertz-Wellen des Streifens *a* ent-wickelt haben mag. Derartige *Frequenzänderungen* konnten bei schädlichen Ein-wirkungen häufig beobachtet werden. Die Amplituden sind nach der sechsten Minute (*d*) stark vermindert, und es lässt sich ausserdem auf Streifen *d* und *e* eine allmähliche Potentialverlagerung nach der elektro-negativen Seite feststellen. Der Streifen *e* zeigt neben steilen Anstiegen, die einen Reizzustand der Ableitestelle andeuten, grösser werdende Pausen. Auf Streifen *f*, 7 Minuten nach Beginn der Registrierung, zeigen sich so gut wie keine Spannungsproduktionen. Hierauf erfolgte die Erwärmung mit körperwarmer physiologischer Kochsalzlösung, und es

ist erstaunlich, dass die darauffolgende Ableitung *g* in der zehnten Minute wiederum das Normalbild, das auch *a* zeigt, ergibt.[1]

Dieser Fall veranschaulicht u. a. die grosse Vulnerabilität der Hirnoberfläche. Die Methodik muss darum bei bioelektrischen Untersuchungen auf der Grosshirnrinde viel schonender sein, als dies bei anderen Experimenten üblich ist.

An früherer Stelle (Kornmüller, 1933 *a* u. *e*) konnten neben anderen Ursachen, die Modifikationen des Normalbildes hervorrufen, noch die *künstliche Hyperventilation* und die *Einverleibung von Hirngiften* angeführt werden.

Reichliche Experimente wurden inzwischen von uns und Mitarbeitern über die Wirkung parenteral einverleibter Stoffe auf das Gehirn angestellt. Ihre *Grundlagen* waren (*a*) *die exakte Lokalisation der Ableitepunkte nach architektonischen Feldern* und (*b*) die von uns beschriebenen *Normaltypen der Eigenströme* (Fig. 2). Sie führten bis jetzt zu folgenden allgemeineren Befunden:

Ein jedes der untersuchten Hirnrindenareale reagierte spezifisch auf die Schädlichkeit. Die Beeinflussung einer Area hängt zur Hauptsache von ihrem Bau und von der Art der Schädlichkeit ab. Bei genügender Erfahrung kann man also unter Umständen aus der modifizierten Feldeigenstromkurve sowohl die Ableitestelle als auch die Art des Giftes erkennen. Auch in quantitativer Hinsicht und bezüglich der Restitution zeigen sich sehr grosse Differenzen unter den einzelnen Hirnrindenfeldern. Es kann gar nicht die Rede davon sein, dass, wie Fischer (1933) schreibt, die Wirkung von Hirngiften eine "verallgemeinerte" sei.

Veit und M. Vogt (1934) haben auf Grund exakter chemischer Untersuchung über die quantitative Verteilung parenteral einverleibter Gifte u. a. die Feststellung machen müssen, dass die Konzentration der untersuchten Pharmaka in der ganzen Hirnrinde mehr oder weniger gleichmässig ist. Die Unterschiede in der klinischen Wirkung führen diese Autoren auf eine ungleiche Vulnerabilität der einzelnen Hirnrindenfelder zurück. Dieser Erklärung im Sinne der Patoklisenlehre C. und O. Vogts (1922) müssen wir durchaus beipflichten. Einzelheiten über die Giftwirkungen, über Narkose und lokale Hirnbeeinflussungen werden von unseren Mitarbeitern (R. Range und M. Gozzano) und uns später berichtet.

Die verschiedensten Reize endogener und exogener Natur rufen *Krampfströme* hervor, über die wir im vorangehenden schon einiges mitgeteilt haben. Die Bilder dieser Krampfströme sind ausserordentlich vielseitig. Alle möglichen Frequenzen können sich abwechseln oder aber auch superponieren. Es ist hier unmöglich alle uns bereits bekannten Typen zu beschreiben oder gar abzubilden. Wir verweisen auf unsere früheren Arbeiten. Fig. 9 stellt einen solchen Krampfstrom in fortlaufender Reihenfolge dar. Lediglich zwischen Streifen *a* und *b* sind 7 Sekunden und zwischen *f* und *g* 6 Sekunden ähnlichen Kurvenverlaufs für die Abbildung ausgelassen. Auf Streifen *a* dieser Figur ist ein normaler Feldeigenstrom der *Area striata*, der durch 6 aufeinanderfolgende Augenbelichtungen des Tieres von entsprechenden Aktionsstromschwankungen überlagert wird. Auf Streifen *b* werden nun durch die ersten der 7 Augenbelichtungen Aktionsstromschwankungen ausge-

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[1] Nebenbei bemerkt ergab *Scopolamin* parenteral natürlich innerhalb von Stunden ähnliche Stadien. Nicht beobachtet wurde lediglich das Stadium des Streifens *e* der Fig. 8.
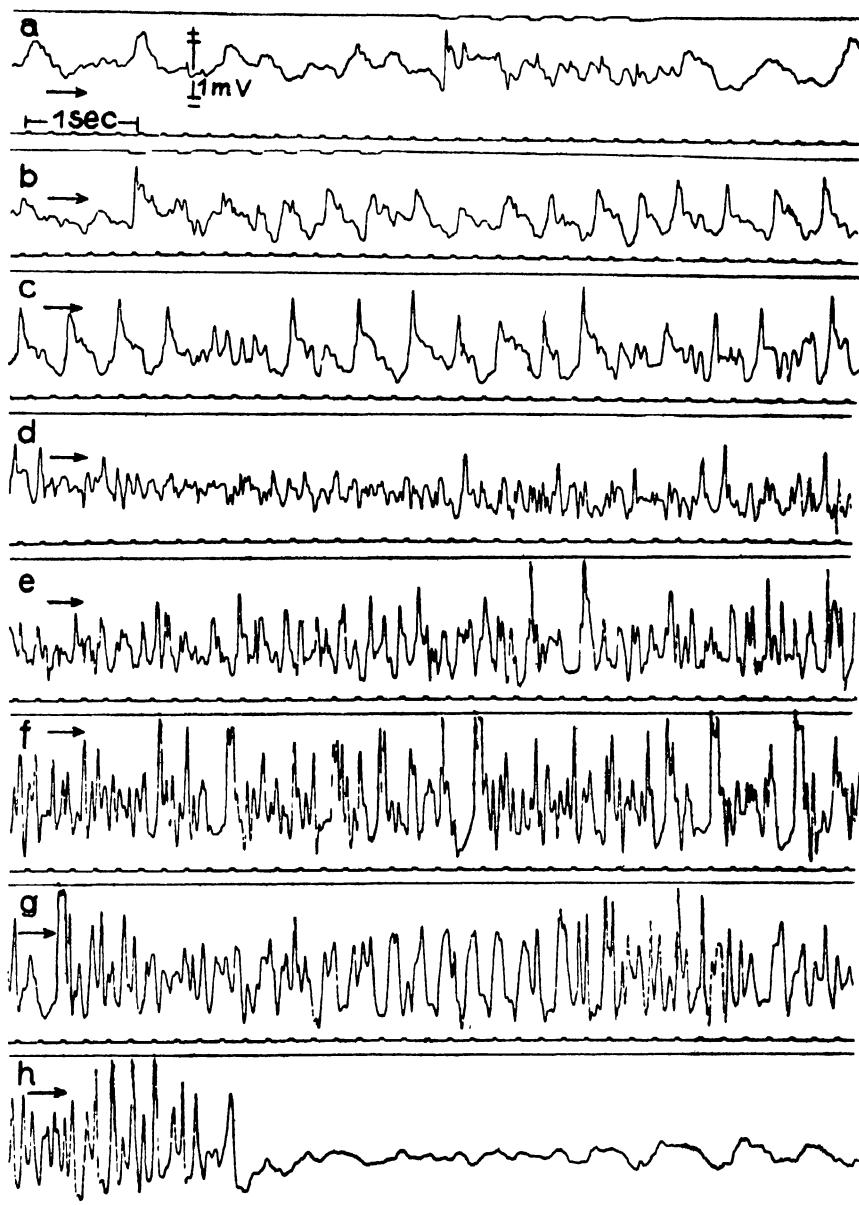
Fig. 9. *a*, Normales bioelektrisches Bild der *Area striata* des Kaninchens (*Str* der Fig. 2). Die Reizmarkierung oberhalb der Kurven von *a* und *b* bedeuten rhythmische Augenbelichtung. Die Augenbelichtung bei *b* ruft einen zunehmenden Krampfstrom hervor, der auf *h* sein Ende findet. Nach kurzer Erschöpfung allmähliches Einsetzen der Feldeigenströme. Registriert mit dem Neurographen.

löst, die von Schwankungen gefolgt werden, welche rascher aufeinanderfolgen, als der Feldeigenstrom (Streifen *a*) zeigt. Es handelt sich um einen durch einen Sinnesreiz ausgelösten Krampfstrom, der in den folgenden Registrierungen immer mehr zunimmt ("Reflexepilepsie"). Man beachte sowohl die Vergrösserung der Amplituden als auch die enorme Frequenzsteigerung! Auf Streifen *h* zeigt sich plötzlich ein starkes Abnehmen der Stromschwankungen. Die Spannungsproduktionen werden auf einmal kleiner als die des normalen Feldeigenstromes. Dies ist wohl so zu verstehen, dass die betreffende Ableitestelle nach den vielen bioelektrischen Entladungen in einen Erschöpfungszustand gekommen ist. Im Laufe der weiteren Registrierung zeigt sich eine allmähliche Zunahme der Amplituden, durch welche sich eine Erholung der Ableitestelle ausdrückt. In diesem Fall wurde von der *Area striata*, also einem Sinnesfeld, abgeleitet. Bei Ableitung von motorischen Feldern waren meistens während der Dauer der Krampfströme motorische Krämpfe zu beobachten. Es ist anzunehmen, dass diese motorischen Krämpfe von der Hirnrinde ausgelöst wurden. Bekanntlich gibt es, wie wir seit H. Jackson wissen, eine kortikale Epilepsie. Aber nicht nur die Frage der Epilepsie, sondern auch unsere Kenntnisse über manche Hirnerkrankungen konnten ebenso wie *therapeutische* Fragen schon jetzt durch die bioelektrische Methode Förderung erfahren.

Da, wie eben gezeigt, schon geringste Einwirkungen zu Modifikationen des normalen bioelektrischen Bildes führen, war es von Interesse zu kontrollieren, welchen Einfluss die üblichen Methoden der Hirnphysiologie auf das bioelektrische Bild und somit auf die Tätigkeit des Gehirns haben.

Die Exstirpationsmethode. Am Kaninchen wurden nach Kontrollregistrierungen der normalen bioelektrischen Erscheinungen verschieden grosse Exstirpationen der Hirnrinde vorgenommen und sowohl unmittelbar danach als auch nach längerer Zeit, bis vier Wochen, die bioelektrischen Erscheinungen studiert. Dabei stellte sich heraus, dass schon bei kleinsten Verletzungen nicht nur unmittelbar nach dem Eingriff sondern auch nach Wochen bis in weite Entfernung von der Exstirpationsstelle stärkste bioelektrische Modifikationen zu beobachten sind. Gleich nach der Exstirpation sind an der exstirpierten Stelle und der näheren Umgebung meistens keinerlei Ströme zu registrieren, oder aber es konnten Krampfströme abgeleitet werden. In weiterer Umgebung, manchmal nahezu über der ganzen gleichseitigen Hemisphäre, waren deutlich Verminderungen und eindeutige Modifikationen der Stromschwankungen festzustellen. Der letztere Befund blieb auch so lange nach der Exstirpation (bis zu vier Wochen), wie das bioelektrische Verhalten kontrolliert wurde.

Demnach besteht für uns kein Zweifel darüber, dass weit über die exstirpierte Stelle hinaus die normale Tätigkeit der Hirnrinde geschädigt bezw. abgeschwächt oder aufgehoben wird. Es ist hier leider nicht möglich, auf Grund des genannten Befundes zu den Untersuchungen und Arbeitshypothesen einiger Autoren, die sich der Exstirpationsmethode bedienen, Stellung zu nehmen.

Die reizphysiologische Methode. M. Vogt und Kornmüller haben in einer längeren Versuchsreihe (unveröffentlicht) die reizphysiologische Methode und die

bioelektrische kombiniert. Unter anderem hat sich dabei ergeben, dass elektrische Rindenreizungen, die zur Auslösung einer motorischen Reaktion notwendig sind, nachträglich[1] Modifikationen hervorrufen, wie wir es weiter oben von verschiedenen exogenen und endogenen Reizen beschrieben haben. Kam es durch starke elektrische Reizung zu der bekannten "Rindenepilepsie", so konnten stets Krampfströme registriert werden. Marthe Vogt konnte darüber hinaus die Feststellung machen, dass in gewissen Fällen weitgehender Synchronismus zwischen Perioden dieses Krampfstromes und den mechanisch registrierten Krampfzuckungen besteht. Voraussetzung dafür sind besonders einfache rhythmische Verhältnisse auch der motorischen Reaktionen.

Die von W. Trendelenburg (1910, 1911, 1923) weiter entwickelte *Methodik der Ausschaltung bestimmter Hirnteile durch Kühlung* hat durchaus den gewünschten Effekt, sie muss auch als schonend bezeichnet werden. Die Möglichkeit der vollkommenen Restitution durch darauffolgende Wärmung kann bioelektrisch bestätigt werden. Siehe Fig. 8.

Die *Methode lokaler Reizung durch Anwendung von Strychnin* (Baglioni, 1909; Amantea, 1921; Dusser de Barenne, 1916, 1933; u. a.) fand neuerdings gründliche Bearbeitung durch unseren Mitarbeiter M. Gozzano. Dessen eingehende Untersuchungen zeigten neben anderem den feineren Wirkungsmechanismus des Strychnins auf das Zentralnervensystem. Strychnin bewirkt auch bei lokaler Anwendung abnorme bioelektrische Erscheinungen. Die Auswertbarkeit der Befunde für das Lokalisationsproblem ist nur beschränkt möglich. Die Kombination der Strychninmethode mit bioelektrischen Untersuchungen (M. Gozzano) eröffnet aber viele neue Perspektiven, so z. B. eine weitere Möglichkeit der Feststellung von Wechselbeziehungen zwischen verschiedenen Teilen des Zentralnervensystems, indem nach vorangehender Strychninisierung eines Gebietes die dadurch hervorgerufenen abnormen Erregungen in anderen Gegenden festgestellt werden können, die mit dem strychninisierten Gebiet in Beziehung stehen (Gozzano).

(e) Anhang: Über die physiologische Reifung des Gehirns.

Am Kaninchen konnten wir *die Entwicklung der elektrischen Spannungsproduktion des Gehirns* studieren. Die einzelnen Versuchsreihen wurden an Tieren eines Wurfes vorgenommen. Das *neugeborene* Tier zeigt keinerlei Stromschwankungen. Die Grosshirnrinde ist also noch gar nicht in Tätigkeit. Dieser Zustand hält einige Tage nach der Geburt an. Am sechsten Tage *post partum* waren kleinste Spuren von Spannungsschwankungen zu registrieren. 10 Tage nach der Geburt betrugen die grössten Spannungsschwankungen nur etwa 0·3 mV. Es hatte den Anschein, dass die bioelektrischen Erscheinungen auf der ganzen Konvexität sich ungefähr gleichzeitig entwickeln. Die Kurvenform hat noch keinesfalls das Aussehen der oben beschriebenen Feldeigenströme. Sie ist aber qualitativ und quantitativ verschieden in den einzelnen Hirnrindenteilen, so dass sich einige Typen unterscheiden lassen. Gewisse Felder, z. B. auch die *Area striata*, zeigten die kleinsten Schwankungen.

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[1] Aus physikalischen Gründen ist eine Registrierung der bioelektrischen Abläufe während der elektrischen Reizung unmöglich.

Andeutungen einer Periodizität sind unverkennbar. Etwa am zwölften Tage liessen fast alle Felder Spannungsschwankungen registrieren. Siehe Fig. 10. Die grössten Amplituden entsprachen etwa 0·5 mV. Manche Typen zeigten eine leichte Ähnlichkeit zu denen des reifen Gehirns (z.B. *Rsg β*). An anderer Stelle wollen wir weitere Befunde über die physiologische Reifung mitteilen und vor allem die Beziehungen dieser zur Myelo- und Cytogenese untersuchen.[1] Hier wollten wir das genannte Problem nur anschneiden und als ein weiteres Beispiel bringen, das durch unsere bioelektrische Lokalisationsmethode gefördert werden kann. Es sei noch darauf hingewiesen, dass eine Reihe von Untersuchungen über die kortikalen Reaktionen auf elektrische Hirnrindenreizungen bei neugeborenen Tieren vorliegt (Lit. bei Graham Brown, 1927). So hat Tarchanow (1878) gefunden, dass die motorischen Hirnrindenzentren des Kaninchens einen elektrischen Reiz nicht vor dem 12. Tage nach der Geburt mit motorischen Reaktionen beantworten.

(4) ANHANG: DIE BIOELEKTRISCHEN ERSCHEINUNGEN DES MENSCHLICHEN GEHIRNS.

Seit langem haben wir das Bestreben, die im Tierexperiment gewonnenen Ergebnisse für die Physiologie und die Pathologie des *menschlichen* Gehirns auszuwerten. Dazu gibt es zwei Wege: (1) *direkte Ableitungen am Menschen* und (2) *indirekte Schlussfolgerungen auf den Menchen* auf Grund der Ergebnisse an Tieren, vor allem an Affen, unter Zuhilfenahme der Tatsachen der vergleichenden Hirnrindenarchitektonik.

Berger hat in grundlegenden Untersuchungen das Elektrenkephalogramm, *EEG*, beschrieben, das durch *bipolare* Ableitung vom uneröffneten Schädel des Menschen mittels Elektroden, die durch die Kopfschwarte eingestochen werden, gewonnen wird. Dieses besteht im wesentlichen aus Schwankungen von 10 Hertz Frequenz (α-Wellen) und kleineren Amplituden doppelter Frequenz (β-Wellen), so dass sich Bilder wie etwa 1 und 7 der Fig. 11 ergeben. Das *EEG* zeigte das gleiche Bild über dem ganzen Schädel, es ergab also keinerlei lokalisatorische Befunde. Abweichungen von dem beschriebenen *Normal*bild hat Berger mit klinischen und teilweise mit psychiatrischen Befunden in Zusammenhang gebracht.

Fig. 11 stellt Stromschwankungen dar, die an normalen Menschen von der Kopfhaut in der Nähe des Scheitelknotens abgeleitet wurden (Tönnies, 1934 *b*). Dieser Autor legte aber die zweite Elektrode an eine Stelle der Haut, die selbst keine Schwankungen, auch nicht des Elektrokardiogramms, aufweist. Dieser Forderung hat der Genannte dadurch entsprochen, dass er als indifferente Elektrode zwei Elektroden, die in Parallelschaltung an den Ohrläppchen befestigt waren, verwendete. Unter diesen Bedingungen ergibt sich, was Fig. 11 zeigt, dass zwar manchmal 10 Hertz-Wellen vorherrschen (1 u. 7), dass aber auch andere Frequenzen häufig beobachtet werden können. Bei den etwa 30 untersuchten normalen Menschen konnte festgestellt werden, dass bei den Personen, die 10 Hertz-Wellen vom Scheitel registrieren liessen, Wellen gleicher Form vom ganzen Hirnschädel

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[1] Die Untersuchungen sind noch nicht abgeschlossen, doch hat es den Anschein, dass die Entwicklung der Spannungsproduktion zur Myelogenese kaum Beziehung hat.
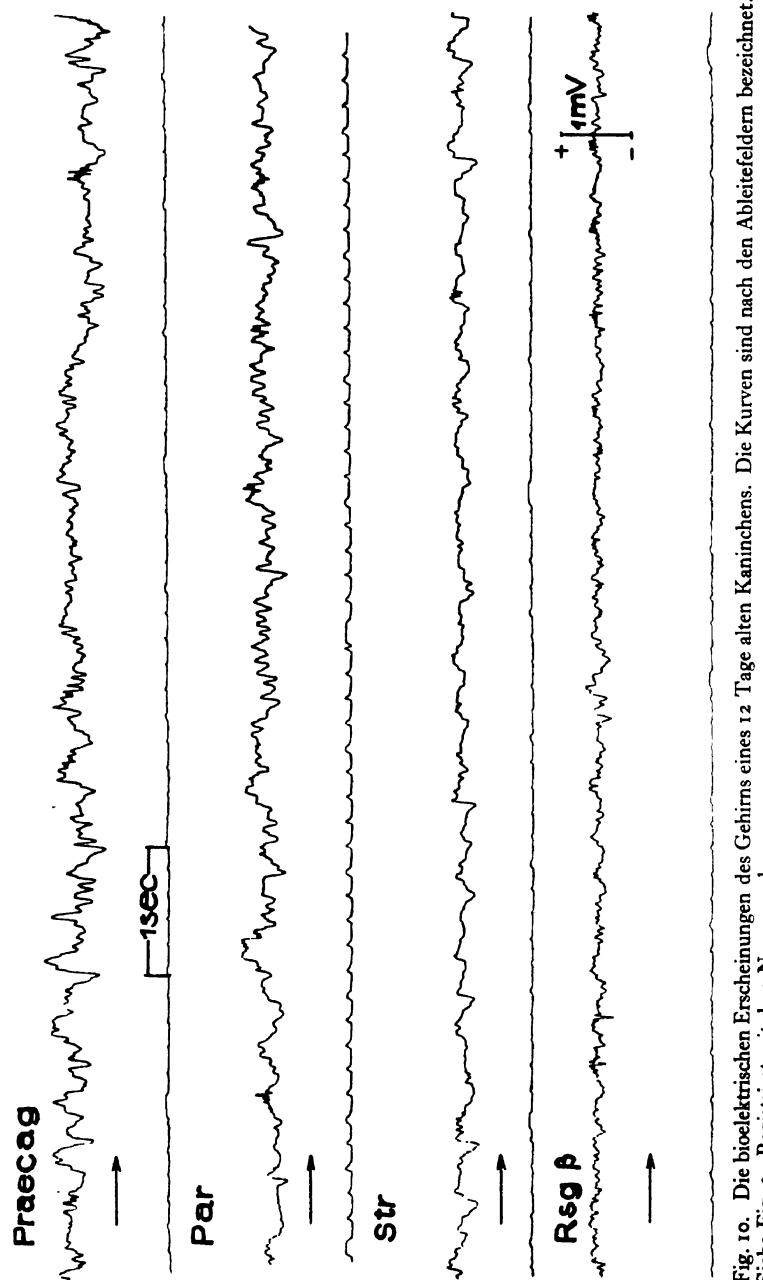
Fig. 10. Die bioelektrischen Erscheinungen des Gehirns eines 12 Tage alten Kaninchens. Die Kurven sind nach den Ableitefeldern bezeichnet. Siehe Fig. 2. Registriert mit dem Neurographen.

abzuleiten sind. Bei anderen Versuchspersonen konnten gelegentlich von verschiedenen Kopfhautstellen verschiedene Frequenzen zur Registrierung gelangen. 2 *a* der Fig. 11 stammt vom Scheitel und 2 *b* von der Stirn derselben Versuchsperson. Auf 2 *a* sind 11 und auf 2 *b* 22 und 18 Hertz vorherrschend. Voraus-
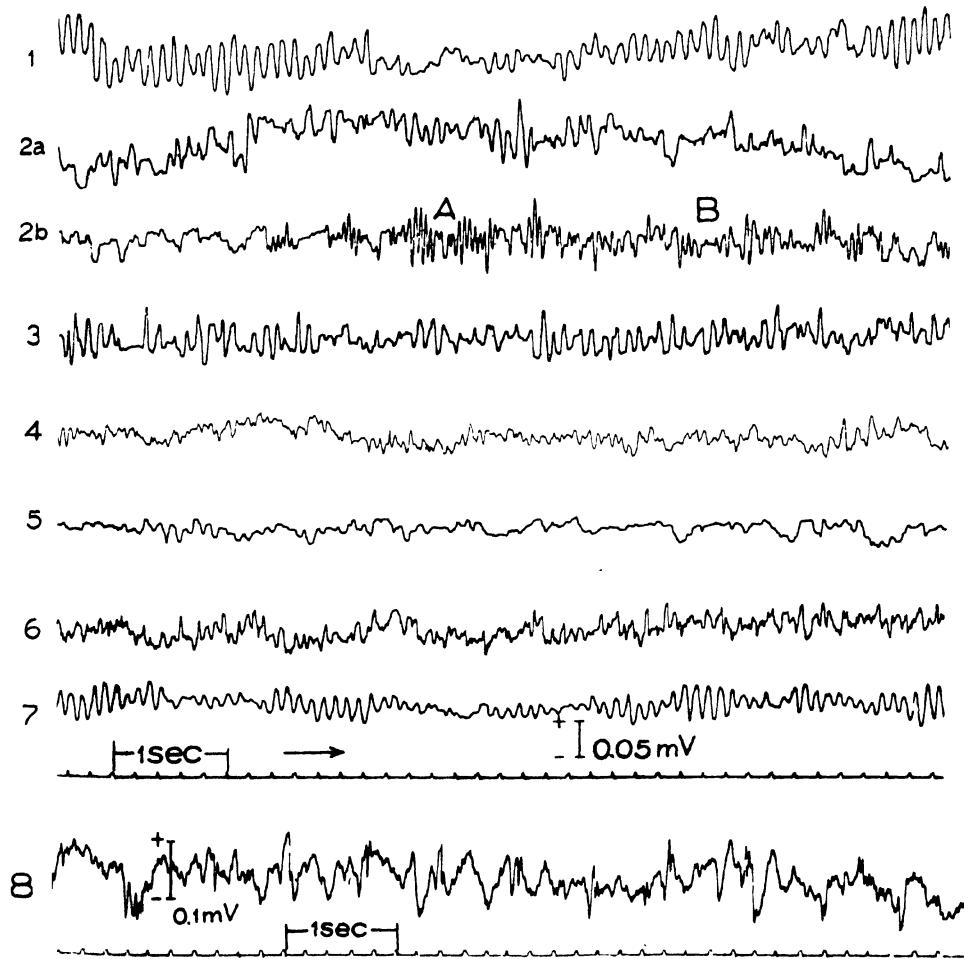
Fig. 11. 1–7, Die Potentialschwankungen von 7 Versuchspersonen bei unipolarer Ableitung von der Haut des Scheitelknotens (Tönnies, 1934). 8, Direkte Hirnableitungen vom Fusse der zweiten Stirnwindung des Menschen. Registriert mit dem Neurographen.

setzung für dieses Ergebnis war die *unipolare* Ableitung. Gleichzeitige unipolare Ableitung von den genannten zwei Schädelgegenden, wobei die Registrierung mit zwei voneinander unabhängigen Apparaten vorgenommen wurde, erbrachte eindeutig das Ergebnis, dass bei dieser Versuchsperson die Stromschwankungen der Stirn nicht synchron mit denen des Scheitels verlaufen. In anderen Fällen konnte

derartiges nicht gefunden werden, was durch ausgedehnte Experimente (Kornmüller, 1933 *d* und Tönnies, 1933 *b*) an Kaninchen, Katzen und Affen verständlich wird. Diese Versuche galten der Beantwortung der Frage, wie weit es möglich ist, durch den uneröffneten Schädel zu lokalisatorischen Daten mittels der Hirnströme zu gelangen. Dabei musste festgestellt werden, dass schon durch den Knochen eine schwache, durch das Periost und die Kopfschwarte aber eine ganz grosse Streuung und seitliche Ausbreitung der Hirnströme erfolgt. So waren z. B. am Affen die Aktionsströme auf Augenbelichtung, die bei direkter Ableitung vom Gehirn nur am Okzipitallappen (*Area striata*) zu finden sind, bei Ableitung von der Kopfschwarte auch noch über der Stirn nachweisbar. Auf Grund solcher Tatsachen bestand von vornherein wenig Hoffnung, auf der Kopfschwarte je nach Lokalisation unterschiedliche Stromabläufe zu registrieren, wie wir es vom Gehirn direkt auf Grund der Ergebnisse an Tieren zu erwarten haben. Die besagten Tierexperimente aber lassen es nicht für ausgeschlossen erscheinen, dass man nach weitem Zurückschlagen der Kopfschwarte und des Periosts durch Ableitung vom freien Schädelknochen bis zu einem gewissen Grade lokalisierte Stromtypen auch am Menschen erhalten könnte.[1] Wegen der nutritiven Funktion des Periosts sind solche Experimente aber bedenklich und darum kaum durchführbar. Durch das freundliche Entgegenkommen von Herrn Prof. Heymann, Berlin, kam Tönnies in die Lage, gelegentlich einiger Hirnoperationen direkte Ableitungen am Menschengehirn vorzunehmen. Eine solche Registrierung vom Fusse der zweiten Stirnwindung zeigt Reihe 8 der Fig. 11. Die Kurve hat wohl kaum eine Ähnlichkeit mit dem Elektrenkephalogramm von Berger, sieht dagegen den Kurven sehr ähnlich, die wir mit Tönnies vom Affenhirn registrieren konnten. Schon dies berechtigt zur Hoffnung, auf dem Wege der vergleichenden Elektrophysiologie und Architektonik der Hirnrinde aus Experimenten an Tieren, vor allem Affen, Schlussfolgerungen auf den Menschen ziehen zu können. Die Möglichkeit, am Menschenhirn direkte Ableitungen vorzunehmen, bleibt gering, und so ist wohl der beste Weg der, auf Grund gründlicher Kenntnisse über die Elektrophysiologie der Hirnrinde des Affen bei Gelegenheit von Hirnoperationen die Vermutungen über das Verhalten des Menschenhirns zu prüfen.

Ohne Zweifel ist unsere bioelektrische Lokalisationsmethode in der Lage dem Hirnchirurgen und überhaupt dem Kliniker wertvolle Dienste zu leisten für die topische Diagnostik von Hirnerkrankungen, vor allem wenn diese die Hirnoberfläche betreffen. Durch unipolare Ableitung lassen sich Abgrenzungen von funktionsgestörtem Gewebe gegen die gesunde Umgebung ermöglichen. So zeigt Fig. 12 oben die nur geringen Potentialschwankungen bei Ableitung von einem Hirntumor (Gliom). Darunter sind die viel stärkeren Stromschwankungen des benachbarten gesunden Hirnrindengewebes (Tönnies[2]). Eine Voraussetzung zur Beurteilung feinster Modifikationen ist die Kenntnis der normalen Stromschwankungen des Menschenhirns. Ströme von extrem abnormem Verlauf (z. B. Krampf-

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[1] Vergl. Nachschrift, S. 426.
[2] *Verhandlungen der Gesellschaft Dtsch. Nervenärzte*, München, 1934. Erscheinen (1935) Berlin, F. C. W. Vogel.

ströme) können wir bereits jetzt für die Beurteilung des pathologischen Zustandes der betreffenden Hirnstelle verwerten. Unter Berücksichtigung unserer diesbezüglichen Vorversuche wird sich möglicherweise doch noch eine Methodik finden lassen, um den Sitz von Hirnrindenerkrankungen, z. B. Tumoren, ohne Trepanation festzustellen. Wir stellen uns vor, dass die Lösung dieser Aufgabe durch Ableitung aus feinen Punktionslöchern ermöglicht wird, nachdem man die leitenden Verbindungen des Gehirns mit dem Knochen und der Kopfschwarte durch Liquorentlastung verringert oder gar teilweise aufgehoben hat. Wie erwähnt, wird ja die exakte Lokalisation von Hirnströmen bei Ableitung von der Kopfhaut durch die seitliche Streuung der Ströme in der Kopfschwarte und dem Knochen sehr erschwert.
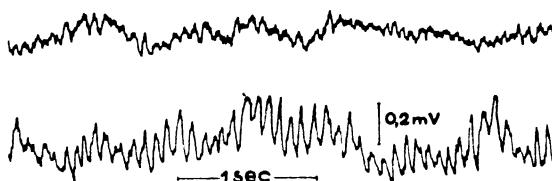
Fig. 12. Oben Potentialschwankungen von einem Tumor der Hirnrinde. Unten · Die Ströme der benachbarten gesunden Hirnrinde (Tönnies). Registriert mit Tönnies' Cordiscriptor.

IV. ARBEITSHYPOTHESE UND GRUNDSÄTZLICHES ZUM STUDIUM DER BIOELEKTRISCHEN ERSCHEINUNGEN DES ZENTRALNERVENSYSTEMS.

Zu einem tieferen Verständnis für die bioelektrischen Erscheinungen der Grosshirnrinde zu kommen, bemühen wir uns schon seit Jahren. In früheren Arbeiten (Kornmüller, 1933 a, 1933 e) wurde bereits eine Arbeitshypothese entwickelt. Fragestellungen physikalisch-chemischer Art über das Wesen der bioelektrischen Erscheinungen erscheinen uns verfrüht, wenn man bedenkt, wie wenig die Natur der bioelektrischen Erscheinungen des peripheren Nervensystems bis jetzt geklärt ist. Zur Zeit gilt es, sich darauf zu beschränken, die Form der Spannungsschwankungen zu analysieren. Drei Hauptfaktoren halten wir dabei für wesentlich : (1) den *architektonischen Bau* der Ableitestelle mit besonderer Berücksichtigung der *Synaptologie*, (2) deren *Erregbarkeitszustand* und (3) die *physikalischen Aussenbedingungen* des Versuchstieres.

(1) Auf Grund von Tatsachen brachten wir (Kornmüller, 1932 und 1933) zum Ausdruck, dass die von der Grosshirnrinde abgeleiteten bioelektrischen *Spannungsproduktionen* zur Hauptsache *von den Ganglienzellen* produziert werden. Nicht ausgeschlossen ist allerdings, dass auch die in der Hirnrinde gelegenen Markfasern und Dendriten der Ganglienzellen, wenn auch nur verhältnismässig kleine Spannungsproduktionen aufweisen. Die von der freien Hirnoberfläche registrierte Kurve ist das *Summationsbild* der elektrischen Spannungsproduktionen aller jeweils tätigen Ganglienzellen der Hirnrinde, die sich unter der Elektrode befinden. Wie schon eingangs dargelegt, zeigen die Ganglienzellen eines Rindenquerschnittes nicht einen gleichen Bau, sondern sie sind nach Schichten verschieden. Siehe Fig. 1 und Abschnitt I, 3. Oben mitgeteilte Befunde legen die Annahme nahe, dass

der Anteil verschieden gebauter Zellen nicht ein gleicher, sondern je nach dem Bau ein differenter und spezifischer ist. Könnten wir, was technisch unmöglich ist, von einer isolierten Pyramidenzelle ableiten, so wäre zu erwarten, dass deren Spannungsproduktion eine andere ist als die einer Körnerzelle, die bekanntlich viel kleiner ist und einen anderen Bau aufweist. Da die elektrische Leitfähigkeit des Gehirns für die in Frage kommenden Spannungsschwankungen eine geringe sein muss, ist es naheliegend, dass sich auf die Elektrode in erster Linie diejenigen Ganglienzellen auswirken, die dieser sehr nahe liegen bezw. mit der freien Oberfläche durch gut leitende Verbindungen in Beziehung stehen. Diese gut leitenden Verbindungen könnten die Spitzenfortsätze darstellen.

Da die von der Hirnoberfläche registrierbare bioelektrische Kurve ein Summationsbild darstellt, sind als ganz wesentlich die *Synapsen zwischen den einzelnen Ganglienzellen* in Rücksicht zu ziehen. Bekanntlich bewirken die Synapsen eine Verzögerung der Erregungsleitung. Sind bei der Ausbreitung einer Erregung innerhalb des Ableitebezirkes viele Synapsen zu überschreiten, so wird sich die Erregung nur langsam ausbreiten. Die Summation der Spannungsproduktionen der einzelnen Zellelemente muss in gleicher Weise allmählich stattfinden. In diesem Falle wird eine träge Kurve resultieren. Erfolgt die Erregungsausbreitung infolge einer geringen Zahl von Synapsen sehr rasch, so müsste das Summationsbild eine steilere bezw. raschere Kurve ergeben. Diese Annahme steht im Einklange mit der Tatsache, dass Felder mit Pyramidenreichtum viel raschere Abläufe produzieren als Felder, die durch Körnerzellen charakterisiert sind. Die grossen Pyramiden besitzen auf gleicher Strecke viel weniger Synapsen als die kleineren Körnerzellen mit ihren relativ kurzen Dendriten. Sicherlich spielen für die Kurvenform ausserdem die *nervösen Impulse* eine Rolle, die *von anderen Stellen des Zentralnervensystems* her die Ableitestelle treffen. Ausser der *Steilheit* bezw. *Trägheit* lassen sich an den bioelektrischen Kurven noch andere Charakteristika unterscheiden, z. B. die *Amplitudengrösse*, die unseres Erachtens eine Funktion der *Zahl der Elemente*, welche eine Zustandsänderung erfahren, ist. Weitere leicht fassbare Merkmale der bioelektrischen Kurve der Hirnrinde sind u. a. die *Frequenz*, die *Periodizität* und der *Grad der Regelmässigkeit* der Abläufe.

Bei der Kompliziertheit des Baues des Hirnrindenquerschnittes und der Mannigfaltigkeit dessen Verknüpfung mit anderen Teilen des Zentralnervensystems *geht es* unseres Erachtens aber *keinesfalls an*, diese morphologischen Tatsachen ganz zu vernachlässigen und selbst nicht einmal *die Tatsache der architektonischen Felderung der Grosshirnrinde bei bioelektrischen Untersuchungen in Rechnung zu ziehen*. Einzig und allein mit deren Hilfe kann man, wie wir oben zeigen konnten, Gesetzmässigkeiten in dem so komplizierten Bilde der bioelektrischen Phänomene der Grosshirnrinde erkennen. Die architektonischen Felder haben beispielsweise bei dem so häufig untersuchten Kaninchen stellenweise eine Ausdehnung von nur wenigen Quadratmillimetern. Schon bei zwei verschiedenen Elektrodenlagen kann von zwei differenten architektonischen Feldern abgeleitet werden. Ohne Berücksichtigung dieser Tatsache können überhaupt keine Schlussfolgerungen aus den sich dabei ergebenden Kurvendifferenzen gezogen werden. Falls ein Hirnphysiologe ver-

ständlicherweise nicht die Möglichkeiten architektonischer Paralleluntersuchungen haben sollte, um die Ableitestellen exakt zu lokalisieren, so kann er nur dann zu allgemein gültigen Schlussfolgerungen aus bioelektrischen Untersuchungen kommen, wenn er die aufgezeigte feldmässige *bioelektrische Differenzierung* der Hirnrinde in Rücksicht zieht.

(2) Dies alles gilt nicht nur für das Studium am gesunden Gehirn, sondern auch in noch höherem Masse für alle *Untersuchungen an Gehirnen, die unter abnormen Bedingungen stehen* (exogene und endogene Reize der verschiedensten Art und selbst die Bedingungen, die das Tierexperiment schafft). Wenn auch z. B. in der Narkose, wenn nur ein kurzes Kurvenstück betrachtet wird, die Schwankungen schematisch aussehen können, so haben aber länger dauernde Registrierungen gezeigt, dass sich das Gehirn dabei, wie bei allen schädlichen Einwirkungen, in einem sehr labilen Zustand befindet, was die vielen verschiedenartigen bioelektrischen Stadien, die in kurzen Zeiträumen aufeinanderfolgen, zeigen. Eine jede Schädlichkeit trifft nicht das ganze Gehirn gleichzeitig und gleichartig, sondern nach Feldern durchaus verschieden. Zu Schlussfolgerungen über das Geschehen des *normalen* Gehirns ist ein solches Material kaum geeignet.

Zur Erklärung der sehr wechselvollen Befunde unter abnormen Bedingungen möchten wir heranziehen (1) Alteration der Ganglienzellen und (2) deren Synapsen. Diese Alterationen scheinen auch spezifisch zu sein nach der Art der Schädlichkeit. Man könnte sich vorstellen, dass die verschieden gebauten Elemente der Hirnrinde auf ein bestimmtes Gift nicht gleichzeitig und gleichartig, sondern in einer gesetzmässigen Reihenfolge reagieren, vielleicht erst im Sinne einer Übererregbarkeit und dann im Sinne einer Lähmung. Bei den degenerativ wirkenden Schädlichkeiten konnte architektonisch von verschiedenen Autoren (C. u. O. Vogt, M. Bielschowsky, M. Vogt, u. a.) eine schichtenweise Degeneration festgestellt werden. C. u. O. Vogt (1922) haben auf Grund solcher Tatsachen ihre Klisenlehre aufgestellt, nach welcher eine verschiedene Vulnerabilität der einzelnen "topistischen" Einheiten des Nervensystems, z. B. der Rindenschichten, angenommen wird. Auch die Synapsenwirkung der verschiedenen Schädlichkeiten könnte als spezifisch angenommen werden. Selbstverständlich lässt sich dabei nicht eine einzelne Zellschicht isoliert betrachten. Je nach Art deren Verknüpfung mit anderen Schichten und anderen Teilen des Zentralnervensystems wird sich jede Zustandsänderung einer Schicht sekundär auf andere Teile auswirken und umgekehrt.

(3) Die *physikalischen Aussenbedingungen* sind für die bioelektrischen Effekte der Grosshirnrinde ebenfalls zu berücksichtigen. Wesentlich sind ihre Intensität und Qualität und vor allem auch das Intensitätsgefälle. Siehe dazu den Abschnitt über die Aktionsströme.

V. ZUSAMMENFASSUNG.

1. Eindeutig wurden elektrische Ströme von der *normalen* Grosshirnrinde registriert. Wir können zwei Arten dieser unterscheiden: (*a*) ständig vorhandene Ströme, als *Feldeigenströme* (*FES*) bezeichnet, die auch registrierbar sind, wenn alle Aussenreize so gut wie möglich vom Versuchstier ferngehalten werden und auch

keine Bewegungen des Tieres zu sehen sind (Fig. 2); (b) *Aktionsströme*, die die bioelektrische Antwort der Hirnrinde auf einen peripheren Sinnesreiz darstellen (Fig. 5). Ist die Hirnrinde unter unphysiologischen Bedingungen, so treten *abnorme* Potentialschwankungen auf.

2. Die *Ableitungen* wurden *unipolar* von der Hirnrinde vorgenommen, d. h. nur eine (die differente) Elektrode lag dem Gehirn an, während die "indifferente" Elektrode eine Stelle des Kopfes berührte, die sich nach Prüfung frei von Potentialschwankungen zeigte. Die Notwendigkeit einer solchen Anordnung für bioelektrische Untersuchungen auf der Hirnrinde wird dargelegt. Zur Registrierung wurden verschiedene Apparate, vor allem aber der Tönnies'sche Neurograph, verwendet.

3. Die *ständig vorhandenen Ströme* sind nicht gleichartig in den verschiedenen Teilen der Hirnrinde. Wir können eine grosse Zahl von Typen solcher Ströme unterscheiden, wobei ein jeder Typus für je ein bestimmtes Areal charakteristisch ist (Fig. 2). Daraus ergibt sich die Möglichkeit, die Hirnrinde nach den Formen ihrer bioelektrischen Abläufe in viele *Areae* zu gliedern und so eine bioelektrische Karte der Hirnrinde anzugeben. Die einzelnen bioelektrischen Felder unterscheiden sich von einander: (a) durch die Frequenzen ihrer Stromschwankungen und (b) durch die durchschnittliche Grösse ihrer Feldeigenströme.

4. Gemeinsam mit J. F. Tönnies wurden gleichzeitig mehrfache Ableitungen, (a) von einem und demselben Feld und (b) von differenten Feldern, vorgenommen. Über demselben Felde sind die Stromschwankungen in der Regel synchron (Fig. 3). Differente Felder zeigen in ihren Spannungsschwankungen meistens ausgesprochene Dyschronismen (Fig. 4). Siehe auch (10) der Zusammenfassung.

5. Alle bisher untersuchten Fälle zeigen, dass sich die bioelektrischen Felder mit den architektonischen Feldern, die linear und scharf von einander begrenzt sind, räumlich decken (Fig. 1). Die Charakteristika der einzelnen architektonischen Felder beziehen sich auf Differenzen in der Zahl, der Anordnung und der gröberen Form der Zellen und Markfasern der Hirnrinde. Markiert man die Grenze einer Area der Hirnrinde, die einen bestimmten Typ von Feldeigenströmen zeigt, durch einen Einstich in die Hirnsubstanz, so ergibt die mikroskopische Untersuchung immer eine *genaue Koinzidenz der bioelektrischen und architektonischen Grenzen* (Fig. 1).

6. Die erhaltenen Resultate sprechen dafür, dass die bioelektrischen und somit die architektonischen Felder der Grosshirnrinde physiologische Einheiten darstellen. Experimentelle Studien über das "Hirnleben" müssen auch diesen Tatsachen Rechnung tragen.

7. Die Grundlage unserer physiologischen Experimente bilden morphologische Tatsachen (Fig. 1). Zwischen dem feineren Bau der Ableitestelle und dem Bilde der erhaltenen bioelektrischen Kurve können enge Beziehungen festgestellt werden. Somit sind wir in der Lage, zumindest beim Kaninchen, aus dem Bilde der normalen bioelektrischen Kurve die Hauptcharakteristika der architektonischen Struktur der Ableitestelle vorauszusagen und umgekehrt. So zeigen z. B. Felder, die viele Pyramiden- und wenig Körnerzellen enthalten, im allgemeinen die rasche-

sten Stromschwankungen; andere Felder, in denen die Körnerschicht vorherrscht, zeigen zur Hauptsache träge Stromschwankungen (Fig. 2). Die ersteren pflegt man sonst als motorische und die letzteren als vorwiegend sensorische Felder anzusehen.

8. Die *Aktionsströme* sind jene Schwankungen der bioelektrischen Kurven, die über einem bestimmten Areal der Hirnrinde erscheinen, wenn ein Sinnesorgan gereizt wird (Fig. 5). Sie treten aus dem Bilde der Feldeigenströme durch ihre spezifische Kurvenform hervor und zeigen eine strenge zeitliche Koinzidenz mit dem jeweiligen Aussenreiz. Die *Area striata* (die auch auf Grund anderer Untersuchungen als das Hauptfeld der Sehsphäre betrachtet wird) zeigt auf Augenbelichtung die ausgeprägtesten Aktionsströme bei allen bisher untersuchten Tieren. Auf Gehörsreize zeigt das Kaninchen Aktionsströme in unserer *Area temporalis anterior*. Bei der Katze antwortet auf Schallreize das äquivalente Feld (Area 52), das in der Meynertschen Anastomose liegt. Dieses Areal möchten wir darum als Hörsphäre ansprechen, obgleich von den Physiologen bis jetzt allermeist der Schläfenlappen seit Munks Untersuchungen als Hörsphäre viel Bearbeitung und Diskussion fand. Unser Areal stimmt mit dem von Morphologen (C. Vogt und Brodmann) als Hörsphäre angenommenen Gebiet besser überein. Diese Tatsachen erlauben die Anbahnung einer vergleichenden Elektrophysiologie der Grosshirnrinde, welche wohl viele Beziehungen zur vergleichenden Architektonik der Hirnrinde hat (Fig. 5).

9. Die Grenzen eines bestimmten bioelektrischen Feldes, das Aktionsströme zeigt, fallen mit dem Umriss des entsprechenden architektonischen Feldes genau zusammen (Fig. 1 u. 5).

10. Der grösste Teil der Hirnoberfläche zeigt keinerlei bioelektrische Antwort auf einen peripheren Sinnesreiz. Nur wenige Felder beantworteten einen Sinnesreiz mit einer *Beruhigung der Feldeigenströme* (Fig. 6). Dieses Ergebnis spricht— wie alle unsere anderen Befunde—gegen die zur Zeit viel verbreitete Lehre von der Ganzheit des Zentralnervensystems. Wir sind vielmehr der Ansicht—was die Lokalisation von Funktionen betrifft—dass die *spezifischen Funktionen an spezifische Strukturen* des Gehirns *gebunden* sind. Selbstverständlich vertreten wir nicht die Meinung, dass komplexe Funktionsleistungen, beispielsweise psychischer Natur, nur an ein bestimmtes Areal gebunden sein sollten. Weiters konnten wir mit J. F. Tönnies durch gleichzeitige mehrfache Ableitungen von verschiedenen Feldern die Feststellung machen, dass *zwischen einzelnen Feldern gerichtete Wechselbeziehungen* bestehen. Synchronismen zwischen einzelnen Schwankungen verschiedener Felder zeigen uns wohl solche Wechselbeziehungen an. Derartige Untersuchungen an Kaninchen, Katzen und Affen sind bereits durchgeführt.

11. Die von uns aufgedeckten Tatsachen einer weitgehenden *Lokalisation* der bioelektrischen Erscheinungen der Grosshirnrinde sind die Grundlage weiterer Untersuchungen. So werden im folgenden Probleme der Physiologie des Zentralnervensystems kurz behandelt. Der elektrische Energiewechsel des Gehirns zeigt gewisse quantitative Parallelen zu dem Gasstoffwechsel, den andere Autoren für verschiedene Teile des Zentralnervensystems unter verschiedenen Bedingungen

gefunden haben. Wintersteins "Reizstoffwechsel" kann mit Hilfe von bioelektrischen Befunden bestätigt werden.

12. Unsere Untersuchungen ergeben, dass die normale Grosshirnrinde ständig tätig ist, dass aber die verschiedenen Felder spezifische Unterschiede in ihrer Tätigkeit aufweisen (Fig. 2). Manche Felder zeigen eine Periodizität in dem Ablauf ihrer bioelektrischen Tätigkeit. Für einige Fälle konnte eine Abhängigkeit der bioelektrischen Charakteristika vom Gasstoffwechsel festgestellt werden. Die von anderen Autoren erhaltenen Befunde, nach welchen das "tätige Gehirn" keinen fassbar grösseren Unterschied in seinem Gasstoffwechsel hat als das "ruhende Gehirn", werden durch unsere Resultate verständlich gemacht.

13. Einige Tatsachen, die die spezifische Umformung der Erregungen im Zentralnervensystem betreffen, werden angeführt (Fig. 7).

14. Modifikationen der normalen bioelektrischen Ströme unter verschiedenen abnormen Bedingungen wurden auf Grund der verschiedenen normalen Typen der Feldeigenströme studiert. Die verschiedensten endogenen und exogenen Einwirkungen auf das Gehirn konnten mit Hilfe der bioelektrischen Methode in Einzelheiten studiert werden (Fig. 8 u. 9). Die bisher gebräuchlichsten hirnphysiologischen Methoden (elektrische Hirnrindenreizung, Exstirpation und andere) wurden durch die bioelektrische Methodik kontrolliert. Die Physiologie, Pharmakologie und Pathologie des Gehirns dürften ebenso wie die Klinik aus dem Studium der abnormen bioelektrischen Erscheinungen Nutzen ziehen.

15. Weiters wurde am Kaninchen die Reifung der bioelektrischen Effekte des Gehirns untersucht (Fig. 10). Dabei ergab sich, dass die Hirnrinde des neugeborenen Tieres keine bioelektrischen Ströme aufweist und somit inaktiv ist. Die ersten Stromschwankungen erscheinen etwa gleichzeitig auf der ganzen Hirnoberfläche, wenn das Tier mehrere Tage alt ist. Aber diese ersten bioelektrischen Kurven sind mit wenigen Ausnahmen noch sehr verschieden von den ständig vorhandenen Strömen eines reifen Tieres.

16. Eine Auswertung der experimentellen Befunde für die Physiologie und Pathologie des *menschlichen Gehirns* wird vorgenommen. Die Registrierungen von J. F. Tönnies (Fig. 11) während Hirnoperationen am Menschen und diesbezügliche Vorversuche von Kornmüller und Tönnies werden besprochen. Es wurden auch Ableitungen von Hirntumoren und deren Umgebung (Fig. 12) vorgenommen (Tönnies).

17. Eine Arbeitshypothese zur Deutung des Wesens der bioelektrischen Erscheinungen wird entwickelt. Dabei finden besondere Berücksichtigung: (a) der architektonische Bau und die Synaptologie der Ableitestellen, (b) deren Erregbarkeitszustand und (c) die physikalischen Aussenbedingungen. Als grundsätzliche Voraussetzung für bioelektrische Untersuchungen auf der Grosshirnrinde wird die Berücksichtigung der architektonischen bezw. der bioelektrischen Felderung des Cortex hingestellt.

18. In dieser Arbeit wurden nur diejenigen Befunde mitgeteilt, die sich immer wieder reproduzieren liessen und somit als gesichert betrachtet werden können.

VI. SUMMARY.

1. Electric currents have been recorded from the normal cerebral cortex. Two types can be distinguished: (a) *spontaneous field currents* which can be recorded even when all external stimuli to the experimental animal have been eliminated as far as possible and when no movements of the animal are visible; and (b) *action field currents* which are the bioelectric response of the cerebral cortex to a peripheral sensory stimulus. If the cortex is exposed to abnormal physiological conditions, abnormal potential changes arise.

2. The records were made by the unipolar method, *i.e.* only one electrode was in contact with the brain, while the second, or "indifferent", electrode was placed on some part of the head which previous tests had shown to be free from variations of electrical potential. The necessity for such an experimental arrangement is explained. Various types of apparatus were used for registration, but particularly the neurograph of Tönnies.

3. The currents, which are always present, are not uniform in different parts of the cerebral cortex. A considerable number of different types of current can be distinguished, each characteristic of a distinct cortical area. It is consequently possible to divide the cortex up into numerous areas by the forms of the bioelectric curves, and thus to make a bioelectric map. The separate bioelectric fields can be distinguished from one another by (a) the frequencies of their current variations and (b) the average magnitude of their spontaneous field currents.

4. Simultaneous multiple records have been made in collaboration with Tönnies (a) of one and the same field and (b) of different fields. In a single field the current variations are in most cases synchronous, whereas the potential variations of different fields are usually far from being synchronous (see also 10 below).

5. All cases so far studied go to show that the bioelectric fields correspond spatially with architectonic fields which are sharply delimited from one another. The characteristics of the individual architectonic fields consist in differences in the number, arrangement and form of the cells and medullated fibres of the cortex. When the boundary of an area of the cerebral cortex showing a particular type of spontaneous field current is marked by an incision, microscopic examination always shows an exact coincidence of the bioelectric and architectonic boundaries.

6. The results obtained point to the bioelectric, and therefore also the architectonic, fields of the cerebral cortex being physiological units. Experimental researches on brain physiology will be obliged to take these facts into consideration.

7. Morphological data form the basis of our physiological experiments, intimate relations existing between the finer structure of the region from which the current is led off and the type of bioelectric curve obtained. It is thus possible, at least for the rabbit, to deduce the principal characteristics of the architectonic structure of a given region from the shape of its normal bioelectric curve, and *vice versa*. Fields, for example, which contain many pyramidal and few granular cells generally give the most rapid current variations, whereas other fields where the granular layer predominates usually show slow current changes. The former are generally considered to be motor, the latter predominantly sensory areas.

8. The action field currents are those variations in the bioelectric curves which arise over a given area of the cerebral cortex when a sense-organ is stimulated. They can be distinguished from the spontaneous field currents by the specific form of the curves and they show an exact temporal coincidence with the external stimulus. In all animals so far investigated, the area striata shows the most pronounced action currents when the eye is illuminated. The area striata is considered on other grounds to be the chief optical centre. The rabbit shows action currents to acoustic stimuli in our area temporalis anterior. The equivalent field (Area 52), situated in Meynert's anastamosis, answers to sound stimuli in

the cat. We are therefore inclined to regard this area as the acoustic region, although since the work of Munk nearly all physiologists look upon the temporal lobe as the acoustic sphere. Our suggestion corresponds more closely with the views of the morphologists (C. Vogt, Brodmann). These facts open a field of future research on comparative electro-physiology related to the comparative architectonics of the cerebral cortex.

9. The limits of a specific bioelectric area showing action currents always coincide exactly with the boundaries of the corresponding architectonic area.

10. The greater part of the brain surface gives no bioelectrical response to peripheral sensory stimulation. A few areas alone responded to a sensory stimulus by a diminution in spontaneous field currents. This, and all our other results, speaks against the theory of the "central nervous system as a whole". We are of the opinion, on the contrary, that specific functions are localised in specific structures in the brain. Naturally we are not of the opinion that complex functions, such as psychical phenomena, are limited to a single area. We were able to show by simultaneous multiple records, in collaboration with J. F. Tönnies, that correlations exist between different areas. Synchronism between potential changes in the different areas has demonstrated interdependent relations of this nature in rabbits, cats and monkeys.

11. The widespread localisation of bioelectric phenomena in the cerebral cortex which we have found has led on to further investigations. Problems in the physiology of the central nervous system are therefore next discussed. The electrical energy changes in the brain show certain quantitative parallels to the gaseous metabolism which other authors have found for different regions of the central nervous system under various conditions. Winterstein's "stimulatory metabolism" is borne out by bioelectric data.

12. Our experiments lead to the conclusion that the normal cortex is permanently active but that the various areas have specific differences in their activities. Some areas show a periodicity in their bioelectric activities. In certain cases a dependence of bio-electric phenomena on gaseous metabolism was found. The conclusions of certain workers that the active brain has no detectably greater metabolism than the resting brain is made intelligible by our results.

13. Certain facts dealing with the specific transformation of excitations in the central nervous system are mentioned.

14. Modifications of the normal bioelectric currents under various abnormal condi-tions were studied from the standpoint of the different normal types of spontaneous field currents. The mode of action of various external and internal stimuli on the brain were studied by the bioelectric method. The principal methods usually employed in brain physiology, such as electrical stimulation of the cortex, extirpation, etc., were checked by the bioelectric method. The results obtained can be applied to the physiology, pathology and pharmacology of the brain as well as to clinical research.

15. The ontogeny of the bioelectric phenomena of the brain was studied in the rabbit. It was found that the cerebral cortex of new-born animals is inactive, showing no currents. The first potential changes appear almost simultaneously throughout the cortex when the animal is several days old; but the first curves are, with few exceptions, still very different from the corresponding spontaneous field currents of a fully-grown animal.

16. An attempt is made to apply the experimental results obtained to the physiology and pathology of the human brain. Records made during human brain operations are dis-cussed. Currents were also led off from brain tumours and their surroundings.

17. A working hypothesis is developed to explain the nature of bioelectric phenomena. In this attempt special attention is paid to (a) the architectonic structure and the synapses of the area serving as conduction-point, (b) its excitability, and (c) the external physical conditions. A fundamental premise for bioelectric experiments on the cerebral cortex is a consideration of the relation of architectonic to bioelectric differentiation of the cortex.

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NACHSCHRIFT.

Noch unveröffentlichte Registrierungen, die H. H. Jasper mit Mitarbeitern vorgenommen hat, ergaben bis jetzt am Menschen ganz analoge lokalisatorische Differenzen, wie ich sie von Tieren aufgezeigt habe. Die Ableitungen wurden durch die Kopfhaut vorgenommen mittels gut durchdachter Elektroden, die in einem ganz geringen Abstand voneinander aufgelegt wurden. Die erste Mitteilung über diese beachtlichen Untersuchungen ist im Druck (*Archives of Neurology and Psychiatry* 1935).

CHEMICAL REGULATION AND THE HORMONE CONCEPT

By JULIAN S. HUXLEY.

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CONTENTS.

I. INTRODUCTION: HORMONES AND OTHER ACTIVATORS.[1]

DURING the past fifty years, our ideas of the scope of chemical correlation have been enormously extended. Adrenalin was discovered only in 1884, and the word *hormone* not coined until 1906. Since 1900, besides the advances in endocrinology in the narrow sense, we have the discovery of growth hormones and wound hormones in plants, of the chemical transmission of the nerve impulses to the end-organ, of the chemical nature of organiser action in the embryo, of hormones which diffuse instead of being carried in the blood stream, etc.

The purpose of this brief article is twofold. First, to draw attention to the variety of the phenomena now included under the head of chemical correlation, and in particular to the rapid extension of knowledge which has been derived from experimental embryology. And secondly, to propose a reasoned classification and terminology, since at the moment there is no agreement as to the definition and use of various important terms.

The first discovery in this field was that of true hormones—*i.e.* substances which are produced by special tissues, which have as their primary function the exertion of specific physiological effects on other tissues, and are carried in the blood stream.[2] They have accordingly been distinguished as *vascular hormones* (Young, 1934).

[1] Before proceeding further I should like to thank Sir Henry Dale, F.R.S., Prof. R. Goldschmidt, and Mr J. Z. Young for kindly reading the manuscript and for various criticisms and suggestions, and to Dr J. Needham for drawing my attention to Hardy's paper and for other suggestions: further to Dr Greenwood and Miss Blyth of the Animal Breeding Research Department, Edinburgh, for allowing me to quote certain unpublished results of theirs.

[2] The word *hormone* was first employed by Starling in 1906 in reference to the substance *secretin* discovered by Bayliss and Starling in 1902. Starling later (1914) defined a hormone as "any substance normally produced in the cells of some part of the body and carried by the blood stream to distant parts which it affects for the good of the body as a whole".

Schäfer introduced the word *autacoid* to denote substances of this sort which were produced in specific tissues and exerted specific effects, and wished to use *hormones* to include other substances (*parahormones*) with non-specific sites and effects. This terminology, however, has not passed into general use. Schäfer's subdivision of his class of autacoids into *harmazones* and *chalones* according to whether their action is stimulative or inhibitory, has also not met with favour, chiefly because it is unworkable in practice.

However, as this may suggest that they act upon the vascular system, and as it is desirable to include those transported in the lymph (Parker, 1934), the term *circulating hormone*, as Sir H. H. Dale has suggested to me, is more suitable. These are best represented in vertebrates but also occur in some invertebrates, *e.g.* Crustacea (Koller, 1929): here it should be noted that their pigmento-motor effect is not specific but is effective also on vertebrate chromatophores (Koller and Meyer, 1930).

Although true or circulating hormones were the first agents of chemical correlation to be discovered, they are highly specialised. They are specialised in two ways: first, in being produced by special tissues for the special function of influencing other tissues; and secondly, in being carried by the blood stream. As regards the first point, it seems clear that chemical correlation must originally have been exerted by substances formed in the ordinary course of metabolism (metabolites), whose effects on other tissues would thus in the first instance be a by-product. The effect of carbon dioxide in regulating breathing *via* the respiratory centre is a case in point.

For metabolites which help in chemical correlation (*e.g.* carbon dioxide, histamine) Gley (1920) has proposed the useful term *parahormone*.

As regards carriage by the circulation, since the simplest animals when adult and all animals in their early stages possess no circulatory system, chemical correlation must in the first instance have operated by diffusion, not by transport in the blood stream. We may thus speak, rather loosely but conveniently, of *diffusion hormones* as opposed to true or *circulating hormones*. Recently, it has been shown that these may co-exist with circulating hormones in the adult vertebrate. The best known are produced at nerve endings, and have been by some authors styled *neurohormones* (see Dale, 1934), or *neurohumoral substances* (Parker, 1933, 1934).

Broadly, it appears that in the higher vertebrates, acetylcholine is the active substance produced at parasympathetic nerve endings, and adrenalin or an adrenalin-like substance at sympathetic nerve endings (Dale, 1934). In the sympathetic ganglia, however, there is evidence that acetylcholine is produced, and the presumption is that it exerts its effect at the synapses between pre- and post-ganglionic fibres (references in the summary by Dale, 1934). There is also evidence, though not yet so complete, that the release of acetylcholine serves to transmit impulses from motor cerebrospinal nerves to voluntary muscle, and the further possibility that it is also liberated at the synapses of the grey matter of the brain (see Dale, *loc. cit.*).

This would perhaps indicate that the employment of acetylcholine as a chemical transmitter in relation to nervous activity is the more primitive method, and that the employment of adrenalin is a later development associated with the specialisation of the sympathetic system. In this latter connection the well-known fact should be noted that adrenalin is also employed as a true circulating hormone, and that as such it is produced by the adrenal medulla which has a common embryological origin with the sympathetic system: the theoretical implications of this fact are considerable.

Passing to the field of experimental embryology, we find that at a certain stage in development, the germ becomes parcelled out into a number of areas which are each predetermined to differentiate into a particular kind of tissue. It seems clear that each such area is thus predetermined owing to the presence of chemical sub-

stances not found in other areas. This parcelling out of predetermined areas has been called *chemo-differentiation* (Huxley, 1924); and the hypothetical chemical substances involved may be termed *chemo-differentiators*. They differ from hormones in exerting their effect in the same region in which they are produced. This character they share with what Goldschmidt (1923, 1927) has called *intracellular hormones, i.e.* bodies produced by genes and causing the cells which have generated them to differentiate in a certain way. As a general term to cover all these types of substance, the term *activator* is proposed, and will be more fully discussed in § v.

Recent work has shown, first, the existence of gradations between these various types of chemical correlators and activators, and secondly of different lines along which evolutionary specialisation has proceeded.

II. DIFFUSION HORMONES.

Let me take this latter point first, and consider the radius of action of diffusion hormones. As an example of large-radius action, we may take the growth hormone of plants, chiefly known through the researches of Went (see summaries by Snow, 1932 and Went, 1935). The chemical composition of this substance has been worked out by Kögl (1933). The growth-regulator is formed at the top of the coleoptile and stimulates the growth of the elongating region several millimetres below. It apparently exerts its action by increasing the plasticity of the cell-walls in the region where the individual cells are enlarging. The same or a similar substance is formed in root-tips, but exerts a retarding influence on the elongating region of the root. The substance is probably an aliphatic compound. It is transported quite fast, at the rate of 10–15 mm. per hour (24–36 cm. per day). A curious point in this connection is that its transport is polarised: it can readily be transported downwards (morphologically), hardly at all in the opposite or centrifugal direction. Its transport in this and other ways is quite different from that of organic food-materials, although both must be transferred from cell to cell.

An interesting fact for our purpose is that a nearly or quite identical substance is produced by yeast, various bacteria and various moulds, and excreted into the culture medium. The delicately adjusted hormone mechanism in the coleoptiles of higher plants would thus appear to be a specialisation, based upon the widespread existence in plants of a growth-regulating substance, the original action of which may be presumed to be upon other individuals of the same species, upon other cells of the same individual, or upon the same cells in which it is produced. We shall meet with similar cases in animals. Other plant hormones, such as the wound hormones of Haberlandt, we need not discuss, since less quantitative knowledge is available on the subject. They apparently also act by diffusion from cell to cell.

As an example of large-radius action from animals, we may take the neurohormone described by Parker (1934) which is responsible for pigmento-motor effects in certain fish. This hormone normally acts in the near neighbourhood of the nerve terminals where it is produced, but it can diffuse for considerable distances (at least several millimetres) at a very slow rate, about 1 mm. per day.

Now the area of effective action of such diffusion hormones in animals may be reduced in various ways. One method is by the presence in the tissues and body-fluids of substances which alter and inactivate the hormone. This is so with the acetylcholine produced at parasympathetic nerve endings and elsewhere.

This substance is very rapidly hydrolysed into acetic acid and choline by an esterase normally present in the body tissues. (Luckily for the progress of physio-logical knowledge, physostigmine (eserine) will completely inhibit this destructive action of the esterase.)

The action of the acetylcholine is thus restricted to a microscopic area in immediate contact with its site of production. It is by such restriction that this neurohormone can function as a precise "chemical transmitter" (Dale, 1934) of the nervous impulse. The exact method by which similar localisation is effected of the action of adrenalin, the neurohormone operative at sympathetic nerve endings, is as yet obscure.

There appear, however, to be two grades of restriction. Most of the physio-logical effects of acetylcholine itself can be abolished by small doses of atropin. The same applies to most of those effects produced by parasympathetic stimulation for which acetylcholine is apparently acting as chemical transmitter; but in a few cases, e.g. parasympathetic vaso-dilator action, and the action of the vagus on the intestine, atropin has no effect. Dale gives reasons for supposing "that in such cases the nerve impulses liberate acetylcholine so close to the reactive structures that atropin cannot intervene".

An extraordinarily immediate linkage of stimulus, chemical transmitter and effect is also seen in the synapses of the sympathetic (superior cervical) ganglia. Other work (see Eccles, 1934) has shown that in the sympathetic system a single pre-ganglionic impulse will produce a single and corresponding impulse in the post-ganglionic fibres, and further that the time taken to traverse the synapse is extremely short. To quote Dale (1934) once more, "one can only suppose that each impulse must cause the release, in immediate proximity to the ganglion cell, of a minute charge of acetylcholine, which fires off a post-ganglionic impulse and then immediately disappears".

By such means a diffusion hormone has been converted for all practical purposes into a local activator. The theoretically important distinction, however, still remains, viz. that the substance is produced by one cell and acts upon another (which in some cases is one of a different kind of tissue).

It is interesting to reflect that if it were not for the normal presence of the destructive esterase, acetylcholine would be carried round by the blood stream and so become a true circulating hormone. This is not the case, of course, with regard to the large-radius diffusion hormones. Here, the fact that they remain as diffusion hormones and do not pass into the blood stream, or at least not in sufficient concen-tration to act as circulating hormones, must be related to the site of their production, and to some restriction on the quantity produced. In this connection a recent experiment of Greenwood and Blyth (unpublished) deserves mention. They find that when oestrin is injected intra- or subcutaneously into fowls, it will exert only a

local action when the quantity is below a certain limit; furthermore, its effect (upon feather colour and structure) is then graded, being most intense close to the point of injection and shading off to nothing concentrically. When, however, a greater amount is injected, it escapes into the blood stream in sufficient quantities to exert a true hormone effect in all regions of the body.

A very different method is the restriction of the radius of diffusion. Witschi (see his summaries, 1932, 1934) has shown that in parabiotic pairs of frog embryos of unlike sex, when grafted "in parallel" (side by side) the male's gonads produce a substance which inhibits the development of the female gonads in the co-twin. The inhibition is greatest on the side nearer the source of the male hormone, and diminishes with distance. Furthermore, no effect is found in similar parabiotic pairs grafted "in series" (tail to head), where the distance between the male and female gonads is greater. This can only mean that the male substance diffuses out of the testis to form a concentration-gradient.

In urodeles, on the other hand, the female gonads are completely inhibited in grafts of both types—*i.e.* diffusion is complete within the range provided by para-biosis: the substance acts like a true vascular hormone. In free-martins in cattle, the only effect produced is of course by way of the blood stream after establishment of vascular connection between the placentae of the two twins, so that the same female-inhibiting substance produced by the testis here falls under the head of a circulating hormone.

Finally, in pairs of toad embryos, no effect is produced in the female gonads, indicating that here diffusion does not occur at all, but that the hormone is confined to the organ by which it is produced.

The work with amphibia, however, does not only show how wide may be the differences in diffusibility of diffusion hormones in early stages. It demonstrates two further important facts. First, that the failure to diffuse in stages before the establishment of the blood stream may continue into later stages, where in more usual physiological parlance we find the failure of an endocrine organ to liberate its products into the blood stream. This is demonstrated in male toads by the presence of the ovary-like Bidder's organ, the persistence of which into the adult could not occur if the testis were liberating its ovary-inhibiting hormone into the circulation. We have a certain parallel to this in the thyroid of larval urodeles in general, and of axolotls throughout life. This produces its characteristic hormone, but does not liberate it into the blood stream.

III. LOCAL ACTIVATORS.

The second important fact is that we have a link between diffusion hormones and localised chemo-differentiators. It appears certain that the masculine substance which inhibits the ovaries in parabiotic frogs and urodeles (and in unlike-sexed cattle twins) is identical with the substance which is produced in the medullary (masculine) region of the embryo male gonad when in its early, visibly bisexual stage, and causes the atrophy of the cortical or female component, and the differen-

tiation of the rudiment into a testis. The prime function of the substance is thus as a chemo-differentiator: where it happens to be able to leak out, it can act as a diffusion hormone as well. This indicates that no fundamental distinction can be drawn between diffusion hormones and chemo-differentiators, any more than between diffusion hormones and circulating hormones (see summary in Huxley and de Beer, 1934, ch. 7).

Even without this striking case, we should have been driven to a similar conclusion by a comparative review of the facts of experimental embryology. For instance, by chemo-differentiation the dorsal lip (organiser) region becomes determined to differentiate into notochord and mesoderm. At the same time it produces the organiser substance or *evocator* (Needham, Waddington and Needham, 1934) which induces neural plate in any ectoderm into which it diffuses. We do not know whether the organiser substance is identical with that which determines notochordal and mesodermal differentiation—quite possibly not. But we can at least assert that both are formed at the same general stage of development in the same localised area. Similarly the localised chemo-differentiation of the optic cup region goes hand in hand with the production of whatever substance (the same or another) is responsible for the optic cup's capacity to induce lens-formation in epidermis by contact.

While on this subject, we may note that these two diffusion hormones, of organiser and optic cup, have only a restricted capacity for diffusion. Their effects appear to be exerted only on tissues in immediate contact with the tissues by which they are produced. For example, the induced neural plate is co-extensive with the underlying organiser region, and does not overlap it on all sides as would be the case if the organiser substance diffused readily (Marx, 1925).

In earlier stages, however, the "capacity to organise", to use a non-committal phrase, can be transmitted, for a piece of presumptive epidermis engrafted into the organiser region of another individual, even of another species, will become "infected" and can later act as an organiser. It is not known whether this is due to the passage of organiser substance from the host organiser cells to the graft, or whether the graft is influenced to adopt the division rate and metabolism characteristic of the surrounding cells, which result in the grafted cells later producing organiser substance. On general grounds the latter is the more probable: if so, the phenomenon is not relevant to a discussion of hormone action.

The inductive capacity of the organiser region persists even when it has undergone differentiation. For example, in the neurula stage lateral mesoderm (which has of course been formed from the invaginated organiser region) will still behave like an undifferentiated organiser and induce neural plate when engrafted. Presumably the organiser substance (evocator) is still present and still capable of contact diffusion.

We should here also mention the fact of *homoiogenetic induction*, or the capacity to induce or organise tissues of the same nature as the inducing tissue, as against the heterogenetic induction exerted by the organiser and optic cup. So far this is only known in pronephric tubules (Holtfreter, 1933) and neural tube (Mangold, 1929). For instance, on grafting a portion of neural tube into a blastula of the same species, the only induction consists of secondary neural folds.

As to the nature of the substance here operative, we know nothing: perhaps it is a slight chemical modification of the evocator. Its mode of action, however, appears closely similar.

We may further note that chemo-differentiators often occur in a concentration gradient, as evidenced by the fact that fragments from the centre of a chemo-differentiated area have a stronger tendency to differentiate into characteristic organs than fragments of similar size from near the margin (for example, the limb-field of Amphibia—Detwiler, 1918). Whether this concentration gradient is established *ab initio* or is due to diffusion from a centre is not certain, though the former is more probable (see various cases in Huxley and de Beer, 1934, ch. 7).

It is well known that most differentiation in insects occurs locally, without the intermediation of morphogenetic hormones such as are so important in vertebrates. The most striking cases known are those of mosaicism, where a group or groups of cells have different genetic (chromosome) constitution from the body as a whole. These patches are in most cases sharply delimited, even when they differ in regard to sex, so that Goldschmidt (1927) has spoken of the hypothetical masculinising and feminising substances, which must be postulated to account for the facts of sex in insects, as "intracellular hormones". These would thus differ from the ordinary chemo-differentiators of vertebrate embryology in being produced in every cell of the body according to its chromosome constitution, but would resemble them fundamentally in being produced by the same cells on which they exert their specific effect. When, however, the characters concerned are non-sexual, there are of course parallels between vertebrates, insects and other organisms such as plants, for in all these there may exist sharply marked mosaic patches of tissue produced in a heterozygote by the accidental elimination of a chromosome carrying a dominant gene.

One of the most interesting examples of an intracellular activator occurs in the unicellular and uninuclear alga, *Acetabularia mediterranea* (Hämmerling, 1934). The cell here is very large, and shows marked regional differentiation of form and function (there is a stalk, which may be over 10 mm. long, and a reproductive portion or "hat" resembling the pileus of a mushroom, the breadth of which may be almost as great). The single nucleus is confined to one of the rhizoids. Experiment has shown that substances responsible for the morphogenesis of the hat and of the rhizoids are produced by the nucleus and then migrate, the former distalwards, the latter basalwards. On the other hand, considerable (but never complete) morphogenesis may be exhibited by enucleate portions of the stalk, showing that the morphogenetic substances are produced long in advance of the time when they are to act (and also are produced in excess), and must be stable, even in the absence of the nucleus, for a long time—up to several months. The nucleus also produces substances necessary for continued existence, but once more these must be produced in excess, for enucleate fragments may live up to 4 months and over. The effect of the nucleus on viability is independent of its effect on morphogenesis.

These substances are of great interest to us since, although intracellular, they are transported and act on regions remote from the organ which produces them. They are thus true diffusion hormones, although intracellular. The apparent polarised

transport is interesting, and should be compared with the similar phenomenon in regard to the growth-hormone of plants (p. 429).

Returning to insects, it is clear that there must also be regional chemo-differentiators in these animals and other arthropods, which allow certain genes to manifest themselves in one region and not elsewhere. For instance, eye-colour and eye-structure genes can only manifest themselves in the region determined as eye by a regional chemo-differentiator (Wolsky and Huxley, 1934). Similarly the intracellular chemo-differentiation of sex in insects merely allows different genes concerned with sex characters to manifest their effects in different regions previously determined as such (*e.g.* wings, genitalia) by regional chemo-differentiation. We must therefore distinguish rather sharply between *regional chemo-differentiators* concerned with the determination of certain regions of the embryo to differentiate into certain kinds of organs, and *intracellular chemo-differentiators* concerned with the manifestation of various possible genic effects within those regions.

A very interesting case of an intracellular chemo-differentiator with a slight capacity for diffusion is recorded by Whiting, Greb and Speicher (1934) in the parasitic hymenopteron, *Habrobracon*. In specimens with eyes mosaic for the two independent recessives, white and ivory, diffusion occurs of some substance produced by the wild-type allelomorph to ivory from the white region into the ivory region, but not *vice versa*: the wild-type coloration characteristic of the substances produced by the two complementary dominants thus occurs as a band confined to the margin of the ivory region. Similarly in mosaic males of the same animal, in which the two sides of the body differ in each possessing one of the two complementary sex-genes, only one of these appears capable of diffusing, so that partial feminisation is confined to one side of the middle line.

A slightly different case of modification of one tissue by an adjacent genetically different tissue is afforded by the case reported by Dobzhansky (1931) in *Drosophila simulans* gynandromorphs. Here the genetically colourless male parts (carrying the gene for white eye) acquire colour when present with other wild-type tissues in the same individual, and more markedly when actually attached to ovaries composed of wild-type tissue.

A similar but even more striking result has been obtained by Caspari (1933) in the meal-moth *Ephestia*, using grafting. A mutant type (*aa*) possesses red eyes and nearly or quite unpigmented testes as against the black eyes and violet-brown testes of the wild type (*AA*). When an *aa* testis is transplanted into an *AA* host (of either sex) it becomes pigmented. When an *AA* testis is transplanted into an *aa* host it remains pigmented; but the *aa* testis of the host becomes moderately pigmented, and the eyes of the host become black or coffee-coloured, instead of red. These effects occur without any contact; they are sometimes exerted even when the graft has been wholly resorbed. It is clear that *AA* tissues (probably all tissues) must give off into the body fluids a substance (possibly an enzyme or its substrate) which causes unpigmented eyes and testes to form pigment.

We must also mention the remarkable case of the intersexual males of *Lymantria* (Goldschmidt, 1923, 1927), in which when the wings are of mixed male and female

character, the two types form an irregular mosaic. Goldschmidt has shown that the pattern can only be understood on the assumption that the substance responsible for male-type determination of the scales (which in turn determines the male-type pigmentation) is formed at some site in the body and then streams out into the wings along the course of the veins, taking an appreciable time in the process. If the switch-over to female metabolism occurs during this period, all the parts not yet reached by the flow will develop of female type. This remains hitherto as a unique case: the gene-determined substances are here, during a certain period, transported in the fashion of circulating hormones.

We have thus intermediates between local chemo-differentiators, both of the regional and the intracellular type, on the one hand, and diffusion hormones on the other.

IV. OTHER TYPES OF ACTIVATOR.

Experimental embryology also provides us with a good example of a substance intermediate between a diffusion hormone and a parahormone. The perforation of the operculum of frog tadpoles at metamorphosis to allow the passage of the right fore-limb was for years a mystery, ever since Braus (1906) showed that the effect was not, as originally supposed, due to pressure from the developing fore-limb. Recently, however, Helff (1926) has shown that it is due to some substance produced by the underlying gills during their degeneration at metamorphosis. Further, the same effect, though in less intensity, can be exerted by other larval tissues (*e.g.* tail muscles) which are undergoing histolysis at metamorphosis, indicating that the substance responsible is produced as a result of the histolysis, and has secondarily acquired its hormonic morphogenetic function.

This instance also gives us indications as to the phylogeny of ductless glands. We know that many ductless glands that now produce vascular hormones have been converted from organs which originally had other functions. The thyroid gland in cyclostomes is derived from a portion of the endostylar apparatus in the larva. The parathyroids are only found in tetrapoda and are derived from the walls of the larval or embryonic gill-clefts which disappear as such in later life. The thymus appears probably to be homologous with the ectodermal nephridia of amphioxus. The pineal is derived from a portion of an original dorsal visual apparatus: if it has endocrine functions, these have supplanted original sensory functions.

In all these cases, new hormonic functions are taken on by an organ which, in respect of its original function, is degenerating. In this they resemble the larval gills of the anuran tadpole, except that these exert their effect only temporarily, during the actual progress of degeneration. The example, however, shows that substances produced as the result of purely degenerative processes can be utilised for hormonic functions; and it is more than likely that this has actually occurred in some at least of the endocrine organs above cited.

We must also mention the chemical substances which exert a correlative action *via* the external medium. These are, as might be supposed, mainly concerned with sex. Some have to do with the union of the gametes, *e.g.* the agglutinins of Lillie

and later workers, to which, according to Carter, thyroxin is closely related (references in Carter, 1932). It is, to say the least, suggestive that thyroxin should be the major component of a true vascular hormone and also important in promoting the activation of egg and sperm in echinoderms and other marine invertebrates. Substances with a similar function, although the details of action differ considerably, exist in plants (*e.g. Chlamydomonas*, see Moewus, 1933). Others are concerned with sex determination, *e.g.* the substance (probably a metabolite) produced by larger specimens of the chain-forming *Crepidula plana* (Gould, 1917) which causes the masculinisation of small, as yet neuter, individuals of the species. These are clearly a type of diffusion hormone which diffuse into the external medium and exert their correlative effect on other individual gametes or organisms: we may apply the adjective *external* to them.

A substance with a similar action is that formed in the proboscis of female *Bonellia*, which exerts a masculinising effect on the sexually undetermined (or very partially determined) larva. This substance can be extracted and will exert its effects in solution, but normally acts only by contact (Baltzer, 1928, 1931).

There remains to be mentioned one further type of correlative action which does not appear to be connected by intermediate links with other hormone effects, since it depends on a different type of fundamental process. I have to thank Dr J. Needham for drawing my attention to this point. W. B. Hardy (1927) pointed out that at an interface between two phases, forces were brought into play which would orientate neighbouring molecules for considerable distances on either side of the interface. He suggested, as a pure hypothesis, that the tonic and trophic effect of nerves might depend on some "action at a distance" of this nature. Dr Needham suggests that this conception could be generalised, and that a biological field with important effects might be brought into being round a centre which possessed such orientating effects. Such a field would then show a gradient in respect of random or non-random orientation of its component molecules. This is, of course, as yet entirely hypothetical, but should be borne in mind as a possible explanation of the morphogenetic effects subsumed under the empirical concept of "individuation fields" (Waddington and Schmidt, 1933; Waddington, 1934),—*i.e.* those areas in which there exists some form of dynamic equilibrium controlling morphogenesis (see Huxley and de Beer, *loc. cit.* p. 310). Such effects, if they are proved to exist, will be excluded from even the most extended definition of hormones through not being chemical in their basis, even should they be proved to be of functional significance in morphogenesis or other biological correlation-effect. They would fall under the head of physical field-action, and are only mentioned here for the sake of completeness.

V. TERMINOLOGY.

We now come to the urgent question of nomenclature. There is at the moment great confusion in this field. This is due to the co-existence of two main schools of thought. One, represented chiefly by physiologists, wishes to restrict the term "hormone" within the scope of its original definition—*i.e.* to what we have here

called circulating hormones. Other examples of chemical regulation in the adult vertebrate, such as chemical transmission at nerve endings, at first sight appear to be of such radically different nature that the necessity for an extended terminology to include both types has not yet been generally envisaged by workers in this field.

The other school, represented chiefly by general biologists, who are more familiar with transitional and aberrant types of action, wishes to extend the term "hormone" to cover most or all of the phenomena of chemical regulation. Goldschmidt with his "intracellular hormones"—a flagrant contradiction in terms if we adhere to the original definition—is the most prominent example.

The discovery of the numerous transitions cited in this brief review has brought the question into a new phase. Certain principles, however, emerge.

(1) The original definition of hormones was framed exclusively on a functional or biological basis. Substances chemically of the most diverse nature were grouped together under the concept of hormones because they were produced by special tissues with a view to performing special chemical functions in the body.[1] This is not only legitimate, but necessary and fruitful. For instance, it may turn out that some "intracellular hormones" are enzymes. This would not invalidate our including them within an extended definition of hormones. Enzymes are defined chemically, hormones are defined biologically: a given substance may thus fall into both categories.

(2) Recent discoveries have revealed many substances which resemble hormones as originally defined in regard to their function, but not in regard to their mode of transport from site of production to site of action. It is not only legitimate, but desirable, to have a term to include all such substances.

(3) As regards the effects of hormones, all gradations are to be found between functional and morphogenetic action, between reversible and irreversible, between temporary or immediate and continuous or long-term.

(4) The sole basis for an extended definition of hormone-like substances can thus be that they are chemical substances produced to exert specific functions in regard to the correlation or differentiation of the organism.

In other words, we must adopt a biological definition. Biological definitions may of course be of very different degrees of value. Sometimes they may merely conceal our temporary ignorance; in other cases they may have a true and permanent *raison d'être*. The term *vitamin*, for instance, is defined on biological grounds; for a time it played a very useful role. It now seems probable, however, that with the advance of knowledge it will gradually disappear, or be merged into some more general term connoting accessory or protective food factors. Some vitamins (like D) may also fall into the category of hormones, since they are produced in one region of the body and have a specific functional effect upon other regions.

There is no sharp line to be drawn between vitamins and mineral accessories:

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[1] I employ the apparently teleological phraseology to avoid cumbersome periphrasis. All biological adaptation has an apparent teleological aspect (pseudo-teleological). See also Starling's functional definition, p. 427, note 2.

and the functional role of different vitamins is exceedingly various. In fact, the only points of agreement which made it possible to group the various organic accessory food factors together under a common head were that they were elements of the diet, that their deficiency or absence affected health, and that the method by which this occurred was mysterious.

With the term "hormone," on the other hand, the case is otherwise. Here the biological definition is on a true functional basis. It is concerned with what they *do*. This is in a sense teleological, but only in so far as all function has teleological implications. Even with advancing knowledge, the hormone concept, or something closely related to it, will continue to prove useful.

The original definition of hormones, however, contained, in addition to this definition in terms of function, a further statement as to transport. Hormones were first defined as substances of a certain functional type, which are transported in the blood stream. It is clear that biologically this is a much less important basis for a definition; and the growth of our knowledge, briefly summarised in these pages, has made its theoretical inadequacy obvious. However, although inadequate as part of a general definition, it can be profitably employed as a basis of subdivision, as first broadly sketched by Young (1934).

If so, we have three obvious subdivisions, according to whether transport is (1) free to all parts of the body, by some form of circulation of body fluids; (2) restricted, owing to its taking place only by diffusion or similar process; or (3) absent, when the substance acts in the same tissue in and by which it is formed.

The only important question which then arises is whether the term "hormone" itself can properly be generalised to cover all three possibilities. It could easily be stretched to cover a different *means* of transport, and so to include both (1) and (2); but to my mind it would be unwarrantable to stretch it still further to cover the *absence* of transport. It is true that such a distinguished biologist as Goldschmidt has done so, but the concept of action at a distance appears to me to have become irrevocably bound up with the notion of hormones, so that to extend the term "hormone" to cover purely local action without transport would rob it of meaning.

Goldschmidt has also classified hormones, but in a slightly different fashion: (1) first-order hormones, directly produced by the genes, including most intracellular activators; (2) second-order hormones, including most diffusion hormones; (3) third-order hormones, including true or circulating hormones. This, however, attempts to apply a different criterion in definition, viz. immediacy of the relation between the substance produced and the ultimate controllers of the organism's characters, the genes, and so cuts across the functional basis of definition which appears to me to be the only sound one.

Further, in many cases we are not in a position to decide whether a substance is produced directly by the genes or not. As Hämmerling (1934) himself points out, although we can be certain that the morphogenetic substances in *Acetabularia* ultimately owe their origin to the nucleus, we cannot at present be sure whether they are produced as such or attain their final condition as the result of interaction with general or regional constituents of the cytoplasm.

Prof. Goldschmidt in a letter points out to me that the marginal interaction between the two genetically different eye-areas quoted above for *Habrobracon* (p. 434) may be simply due to ferments and their substrates. "Vielleicht sind dabei nicht die Hormone beteiligt, sondern einfach die Chromogene und Oxydase." This again seems to me to be beside the point. Even if the substances concerned were chemically a chromogen and an oxidase, they would also fall into the functional category of intracellular activators, one of which has a slight capacity for diffusion.

The non-committal word *activator* is therefore proposed as a general term. Opinion may still be divided as to whether the word *hormone* should be confined to its original restricted sense, or enlarged to cover all cases in which transport from tissue of production to tissue of action is found. Personally, I favour the extended usage, as the *fact* of transport is biologically much more fundamental than the precise *method* of transport. Further, the current physiological usage of the term neurohormone has already given respectable sanction to the extension.

Our classification will therefore run as follows:

A. *Activators.* Chemical substances produced by the organism which exert specific functions in regard to correlation or differentiation.

I. *Local activators*, with effects on the same tissue or cell by which they are produced.

(*a*) *Intracellular activators* ("intracellular hormones", Goldschmidt) acting in each cell singly. Apparently the direct expression of gene activity, in relation to regional differentiation (and external conditions). Example, the substances responsible for the sex characters of insects, and for many Mendelian characters. These may in certain cases show a slight degree of diffusion, or even pass into the blood stream.

(*b*) *Regional activators* or *chemo-differentiators.* Substances responsible for the pre-determination of specific regions in the embryo, *e.g.* limb-disc, parts of eye rudiment, chick pronephros. These regions often show a gradation of intensity of potency from the centre outwards, indicating a similar gradation of the activator. This gradation may theoretically be due to diffusion, but is more likely to be present *ab initio*, as a result of the presence of field gradients in a previous stage (see p. 433). Substances responsible for growth-gradients form a special subdivision of chemo-differentiators (see Huxley, 1932, ch. 3).

II. *Hormones* or *distance activators*, with effects on tissues or cells other than those by which they are produced.

(*a*) *Diffusion activators* or *diffusion hormones*, transported by diffusion ("tissue hormones", Young, 1934). (i) Effective action restricted only by diffusibility in all directions through the tissues (or into the external medium). *E.g.* pigmento-motor neurohormones in fish, male hormone in frog parabiotic twins. (ii) Direction of transport restricted by structural organisation. Growth-hormones in plants. (iii) Effective action normally restricted to

tissues in direct contact with hormone-producing tissue; *e.g.* organiser substance (evocator) in vertebrate embryo; lens-inducing substance of optic cup; homoiogenetic-inducing substances; masculinising substance of the proboscis in female *Bonellia*. (iv) Effective action restricted by specific means, so that the substances function as quick-action chemical transmitters: neurohormones of higher vertebrates. These last two types may be grouped together as *contact hormones*, and the neurohormones may also be distinguished by Dale's term, *chemical transmitters*.

A different classification of diffusion hormones is into *internal* and *external* (p. 435), according as to whether their action is upon the same organisms by which they are produced, or another.

(*b*) *Circulatory activators* or *circulatory hormones* (hormones as originally defined) transported in the blood stream or lymph, usually to all parts of the organism. These include hormones produced by endocrine glands, and probably certain vitamins and antibodies.

B. *Parahormones and para-activators.* By-products of normal and pathological metabolism with effects on correlation or differentiation: *e.g.* carbon dioxide (effect on breathing); histamin (effect on capillary permeability). These could be further subdivided according to mode of transport, but this would be unprofitable.

It might be objected that to use a special term for normal constituents of the body, like carbon dioxide or water (which latter could presumably be classified as a parahormone for renal function), is a *reductio ad absurdum*. Admittedly this category of substances is ill-defined. It is, however, useful to have such a term for certain cases; further in general it serves to emphasise both the functional definition of hormones and hormone-like substances, and the implication of that definition that, in all cases of the action of such substances, the specific reactivity of the tissue acted upon is of equal importance with the nature of the substance exerting the action.

VI. SUMMARY.

Summing up, our general conclusions are therefore as follows:

1. The term *hormone* can only be defined biologically, as a chemical substance produced by one tissue with the primary function of exerting a specific effect of functional value on another tissue.

2. This biological definition is justifiable, and likely to prove of permanent value.

3. All gradations exist between hormones as above defined and substances which exert their specific effect on the same tissue by which they are produced. The term *activator* is proposed to cover these substances as well as hormones.

4. All gradations exist between hormones and activators above defined, and substances produced as by-products of metabolism (normal or pathological) which then exert effects (of specific functional or of accidental nature) on the other tissues

(or on the same tissue). For these the term *parahormone* (Gley) or *para-activator* will serve.

5. Activators may be classified according to their means of transport: (*a*) *Local activators*, no transport. (*b*) *Distance activators* or *hormones*, with transport; (i) *diffusion hormones*, transport by diffusion, (ii) *circulating hormones*, transport by the blood stream or lymph.

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THE SYSTEMATIC VALUE OF CUTICULAR CHARACTERS IN RECENT AND FOSSIL ANGIOSPERMS

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CONTENTS.

I. INTRODUCTION.

THE detailed study of the cuticles in recent and fossil gymnosperms has made it abundantly clear that in these plants stomatal and other epidermal characters are often of the utmost value in the delimitation of genera and of larger groups, as well as in distinguishing the fragmentary fossil remains of apparently closely allied species. It is unnecessary to review even briefly the results obtained by numerous workers in this field; a fairly comprehensive bibliography covering all groups of gymnosperms may be found in Florin's monumental work on the cuticles of conifers (1931), supplemented by his studies of mesozoic cycads (1933). In the former work (of which only the first part, on Recent conifers, has so far appeared), in discussing the importance of cuticular analysis for the study of fossil plants, with special reference to the conifers, the author incidentally remarks (p. 523) that though a cuticular analysis of the bryophytes and pteridophytes could only exceptionally be of use, since the outer and radial walls of the epidermal cells in these groups are only slightly or not at all cutinised, among the angiosperms on the other hand the occurrence of cutinised remains in deposits of Upper Cretaceous age onwards promises valuable results in a field that is at present almost untouched; the successful application of cuticular analysis to fossil angiosperms demands, however, extensive and detailed studies over a wide range of Recent forms. The colossal task of investigating systematically and completely the whole range of angiosperm cuticles will, one may hope, eventually be undertaken by Dr Florin himself, when he has macerated and described the cuticles of all known gymnosperms. Meanwhile, other workers have touched the fringe of the problem, and

students of Tertiary plants are paying more and more attention to those leaf deposits in which cuticles as well as mere impressions have been preserved. A review of the work so far published on fossil angiosperm cuticles may therefore be useful, especially as doubts have recently been expressed as to whether any value can be assigned to cuticular characters in the angiosperms.

Some palaeobotanists, it seems, though prepared to rely on anatomical characters for systematic distinctions when they are found in petrified coal-balls, reject them when they occur in mummified cuticles, while another attitude is expressed in the words of an American palaeobotanist (now deceased) who, according to report, "guessed he had no time to fool around with cuticles". Gordon (1929, p. 438), while reluctantly admitting that cuticular structures might be of specific value in the gymnosperms, doubted if they were of any use in the angiosperms, and more recently one of his pupils has published a lengthy paper (Odell, 1932) which attempts to prove that the epidermal features of the vegetative parts of angiosperms are unsatisfactory for diagnostic work.

In his presidential address to Section C of the British Association at Aberdeen (1934) Prof. W. T. Gordon again dealt incidentally with this question. His remarks seem to imply that because vegetative characters are not used as the *basis of classification* in flowering plants, therefore they are not safe criteria for the *identification of individual species*. In emphasising the value of floral characters, he seems to overlook the still enormous difficulty of identifying isolated fossil remains of the reproductive organs, whether of flowers or of fruits and seeds; thus out of a total of 314 species of comparatively well-preserved fruits recognised by Reid and Chandler (1933) in the London Clay, fifty-nine could not be referred to a family at all, and a score or more of the other attributions were doubtful. Gordon also states that "the case for the gymnosperms is rather better; we may place some confidence in their determination by vegetative characters, but not flowering plants". The reason for thus making an exception in favour of the gymnosperms is not clear, but it may be suggested that since there are far fewer gymnosperms the case is *easier*, and that their vegetative parts and cuticular structure have been far more intensively studied. The available evidence, summarised in this review, all indicates that given careful and critical work on well-preserved material, together with a detailed comparison with a wide range of living forms, results obtained from a study of fossil angiosperm cuticles will be as valuable as those derived from any other fossil remains, and certainly far more reliable than those founded on leaf impressions alone.

II. RECENT MONOCOTYLEDONS.

An ingenious method of investigating certain epidermal characters was introduced by Molisch (1920), who found that the residue of leaves which had been slowly reduced to ash often showed characteristic figures due to the siliceous skeletons of cell walls or hairs, cystoliths, calcium oxalate crystals, and so on. These *spodograms*, as Molisch called them, often show features which would not

be observable in ordinary cuticular preparations, and are particularly valuable in families such as the Gramineae in which the epidermal cell walls are highly siliceous. Molisch dealt with the general features of the spodograms in several monocotyledonous families and also suggested that in some cases they showed distinctive specific characters. Werner (1928) used the method for distinguishing the various species of Austrian meadow grasses, and of late years some Japanese workers have made extensive studies of the systematic value of spodograms. Ohki (1932) has investigated the Japanese bamboos, and was able to classify all the species examined by means of the spodograms of the leaves. This he considers a highly satisfactory result, since one could scarcely expect any one organ, especially a leaf, to possess all the characters necessary for specific classification. Nevertheless he points out that numerous species of bamboos have not yet been investigated, and that the spodograms of some genera even, such as *Sasa*, *Sasaella* and *Pseudosasa* (which are presumed to belong to the same phylogenetic stock) are not easy to distinguish. Some of the specific differences, too, are very slight indeed. At the same time the bamboos are a very difficult group, and when complete criteria are not available the spodogram method is a simple means of studying microscopical differences between species which are apparently otherwise indistinguishable.

The epidermal characters of numerous genera of Gramineae were described and well illustrated by Grob (1896), and, to take an example of a more recent investigation, Satake (1931, p. 508) has shown that the genus *Hakonechloa*, which is closely allied to, and was formerly included in, *Phragmites*, can easily be distinguished by the structure of the epidermis. The most valuable account of the epidermis of the Gramineae is, however, that recently published by Prat (1932). This author finds that the fixed characters connected with the structure or "aspect" of the elements, or with their mode of distribution, are the most important systematically. He puts aside dimensional characters, because, although instructive in certain cases, the amount of preliminary study of their variability and susceptibility to environmental modification would be prohibitive for taxonomic work. Simple differences of form between homologous cells often provide generic distinctions; those connected with siliceous cells and stomata often show considerable fixity and may be used as characters of tribes. Special epidermal elements, such as hairs of various sorts, long papillate cells, and so on, may characterise groups of the importance of a tribe or subfamily. Purely structural differences are often insufficient to distinguish closely related species or genera; characters drawn from the mode of distribution of the elements may then often furnish a means of discrimination. A classification based on structural characters of the epidermis agrees on the whole with that based on floral morphology, though there are exceptions. In general, genera which differ from the rest of their tribe in epidermal structure also differ in fundamental anatomical characters, thus *Nardus* differs in all characters from the remainder of the tribe Hordeae, and *Eragrostis* from the Festuceae. Two groups of grasses can be distinguished by epidermal characters, corresponding on the whole with the subfamilies Panicoideae and Poeoideae; the tribe Bambuseae, however, approaches the former epidermally, and the latter florally, and since it is

also clearly distinct in certain characters from all the other tribes, it should be considered as an independent subfamily. Prat's paper is well illustrated, but is by no means (and does not profess to be) a complete survey of the whole family; even with the aid of his special terminology, formulae, and schematic diagrams this would be a formidable undertaking.

Prat himself is fortunately continuing the work in a series of papers: that on *Jouvea* (1933) shows from epidermal and other anatomical characters that this genus must be removed from the Hordeae and even from the Poeoideae, thus drawing attention to the imperfections of the older classification based on purely morphological characters. Another paper (1934) discusses the epidermal characters of the Chlorideae and maintains that this tribe belongs to the subfamily Panicoideae. The relative value of the various epidermal characters in the Gramineae are summarised and compared with nuclear and other anatomical characters in a further short note (Prat, 1934 *a*).

Fernald (1932, p. 15) in discussing the diagnostic characters of various organs of *Potamogeton*, says concerning the leaves that "the development or non-development of lacunar systems (rows of nearly empty and colourless cells, presumably giving buoyancy) is one of the most important of characters. Such lacunae are frequent in the floating leaves and in many species with only linear leaves they are often present in abundance in the uppermost or involucral leaves subtending inflorescences. But their regular appearance in or their absence from the truly submersed linear leaves often furnish characters of real value." He proceeds to discuss the distribution of lacunae in various linear-leaved species of *Potamogeton* and points out that closely similar species can frequently be distinguished (in foliage specimens) solely by study of the lacunar system.

As a result of a very detailed study of the leaves in the genus *Echinodorus* (Alismaceae), Meyer (1932) concludes that a whole series of anatomical characters is of value in distinguishing the species. He enumerates over twenty such characters in the stalk or blade of the leaf, including the form, size, height and arrangement of the epidermal cells and the nature of their walls, the distribution and orientation of the stomata, the occurrence and form of trichomes and so on, as well as the structure of the mesophyll and the bundles and the occurrence of oxalate crystals and lacunae. The secretory passages, known as "puncta pellucida" and "lineae pellucidae" provide in the genus *Echinodorus* particularly valuable diagnostic characters. Meyer also concludes that even the varieties might be distinguished by anatomical characters of the leaf. In another paper Meyer (1932 *a*) considers the bearing of leaf anatomy on the question of the possible relationship of the Alismaceae and the Ranales; he finds that the resemblances are mostly of minor importance (similarities in form of epidermal cells and in structure of mesophyll), and are outweighed by differences in the stomatal apparatus (subsidiary cells present in the former, absent from the latter) and in the type of hairs, while the very characteristic secretory organs of the Alismaceae are not found in the Ranales.

Species of *Typha* can also be separated by certain anatomical characters. The form of the epidermal mucilage glands was found to be distinctive in each of four

species described by Meyer (1933), although there were only slight differences in stomatal structure and arrangement of the epidermal cells.

When complete, Solereder and Meyer's *Systematische Anatomie der Mono-kotyledonen* (1928), which is now in course of publication, will doubtless deal as comprehensively with living monocotyledons as the senior author's well-known work does with the dicotyledons.

For an account of the stomatal structure of the palms, the numerous fossil forms of which do not seem up to the present to have yielded much in the way of cuticular remains, see particularly Rudolph (1911) and the references there given.

III. FOSSIL MONOCOTYLEDONS.

Very few fossil monocotyledonous cuticles have so far been described. Unger (1852) gave a diagrammatic figure of the cuticle of a presumed member of the Typhaceae from the Tertiary of Styria, which he named *Typhaeloipum lacustre*. A supposed *Potamogeton* described in the same paper has since been shown to belong to the Loranthaceae (Knoll, 1904; see below). Berry (1920, p. 399) figures the cuticle with stomata of a species of *Pistia* from the Upper Cretaceous of North Carolina, and also briefly describes the cuticle (with a diagrammatic figure) of a less well-preserved leaf, possibly monocotyledonous, which he names *Doryanthites cretacea*. The epidermal cells of a *Potamogeton* (Miocene of Styria) are figured by Hofmann (1926); the same author figures (1930) the epidermis, without stomata, of a palm referred to *Chamaerops* from the Eocene of Geiseltal. Seward and Conway (1934, p. 725) figure the cuticle of a doubtful monocotyledonous plant from the Tertiary of Kerguelen. Stockmans (1932 *a*) deals with the epidermis of *Posidonia perforata* from the Eocene of Belgium. He finds that although it agrees very closely with the epidermis of living species of *Posidonia*, a similar structure is also found in the allied genera *Cymodocea*, *Pectinella*, *Halodule* and *Ruppia*. Certain slight differences among these living forms are perhaps specific rather than generic, and the attribution of the fossil to *Posidonia* depends partly on other characters, such as the entire margin of the leaf.

IV. RECENT DICOTYLEDONS.

For the dicotyledons Solereder's great work (1908) summarises all the earlier observations and is a mine of information to which the palaeobotanist will naturally turn when studying fossil cuticles. Linsbauer's more recent work on the epidermis (1930) may also be mentioned, though the subject is not treated systematically. Solereder's review of those anatomical characters of the leaf which are of taxonomic value (pp. 1070–1173) is particularly helpful in placing the family to which an unknown cuticle may belong, for he deals mainly with the family or sometimes generic distinctions, and is not specially concerned with specific diagnosis. He lays down certain general principles which, one would think, would gain universal acceptance. Thus he speaks of the varying systematic value which attaches to the

individual anatomical characters and says that the guiding principle in systematic anatomical investigations should always be to take all the anatomical features into consideration, and to test their systematic value in each individual case.

The method adopted by Odell (1932) in arguing against the determinative value of epidermal characters, is to take each feature separately, and then, on grounds of variation, or inapplicability in individual cases, to reject it as useless in taxonomy: a line of argument which could obviously be applied to any separate feature either of the reproductive or the vegetative organs. As Hamshaw Thomas has pointed out in this connection (1933, p. 195), plants or plant structures should be compared on the maximum number of available characters, and although individual features, such as the shape of the epidermal cells, may vary within wide limits, the sum of these varying characters must be taken as the basis for diagnostic work. Odell also fails to make any distinction between characters which may be of family or ordinal or even wider importance, and those which may be generic or specific. The absence of subsidiary stomatal cells in a large series of families, the absence of glandular hairs in another series, the presence of three types of hairs together in several families, and similar points which she quotes from Solereder in support of her position, are, on the contrary, unquestionably of value in classification. Her paper collates from various sources a number of interesting observations on the behaviour of cuticles and stomata under different conditions, but many of them have little taxonomic bearing. Her figures for stomatal frequency on p. 952 (varying from 1·25 to 5 stomata per sq. mm.) were clearly incorrect for the genera concerned, and a corrigendum slip gives a revised set of figures, but even these do not accord well with other observations. Thus Odell gives the average number of stomata per sq. mm. in both *Aralia filicifolia* and *Persea linque* as 117 (revised figures); the average of half a dozen counts from cuticle preparations of these species in the British Museum was 337 and 242 respectively. Other figures are, for *Pseudopanax crassifolia*, 110 (Odell), 275 (B.M.); *Ficus elastica* 106 (Odell), 138 (B.M.), and so on. Her attempt to prove the identity in leaf form, venation, and epidermal structure of unrelated pairs of species is scarcely satisfactory. None of these pairs is described, and figures are given of only one of them: *Campanula latiloba* and *Inula salicina* var. *denticulata*. Her own drawings do not suggest identity of epidermal structure, and, in preparations which I have examined from named material in the British Museum herbarium, the two cuticles can even be distinguished by the distribution of the stomata: in the *Inula* the rather sparse stomata (200 or less per sq. mm.) tend to be arranged with their long axes nearly parallel, whereas in the *Campanula*, with 270 or more stomata per sq. mm., they are irregularly scattered. Moreover, the upper epidermal cells of the *Campanula* are papillate, whereas those of the *Inula* are not.

It is unnecessary to go into further detail concerning these cuticles, but a protest must be made against the statement that the leaves of these two species have identical venation. It is true that both have a midrib and secondary veins; otherwise there is little resemblance between them. Moreover, the leaves not only differ in texture but can also be distinguished by an examination of the margin, which in

Inula salicina var. *denticulata*, unlike *Campanula latiloba*, is usually very finely denticulate between the larger teeth.

Kräusel (1933) justly remarks that if, out of eighty-four genera examined, Odell only found four cases of identity, she could scarcely have provided better evidence of the value of the cuticular method. The proportion of "identical" genera may be even lower than this; there may, of course, be cases of unrelated leaves which closely resemble each other in cuticular structure as well as in form and venation, but the evidence is not yet forthcoming. Straus (1933) emphasises the opposite standpoint: that in cases of convergence of form microscopic structure affords the only sure means of distinguishing isolated leaves. A paper on two genera whose leaves are externally similar may be mentioned here: Kubart (1927, p. 590) has pointed out, apropos of a discussion as to whether certain North American Tertiary leaves belonged to *Fraxinus* (Oleaceae) or to *Umbellularia* (Lauraceae), that the structure of the epidermis, if preserved, would settle the matter at once, and he gives figures and descriptions of the epidermis of recent species to illustrate the great differences between the two genera.

On the general question of the use of anatomical characters for systematic purposes reference may be made to a useful summary by Fritsch (1903), who points out, for example, that glandular hairs are of ordinal and perhaps generic rather than specific value, and that papillae on epidermal cells are often an excellent specific character.

The spodogram method mentioned above in the section on monocotyledons has been applied to the Urticales by Satake (1931). His general standpoint is that a natural system of classification "should be based on all the characteristics possessed by plants, including morphological, anatomical, cytological or even physiological ones", and that spodograms may be very useful for distinguishing plants when they are not in flower. He concludes that in spodograms of the Urticales the cystoliths offer the most important criteria, and after them the crystals of calcium oxalate. He considers that plants can probably be classified up to the limit of genera by this means, but that in large genera such as *Ficus* and *Morus* it is impossible to distinguish species by spodograms alone. In *Ficus* itself Satake finds (p. 499) that the leaves of the climbing species have a different structure from that of other groups, and also that there are structural differences between the leaves borne on young stems and those borne on old stems of the same species. A more extended investigation of these points would be of considerable interest.

Spodograms of the Urticaceae have been studied in greater detail by Bigalke (1933) who made preparations of nearly 200 species belonging to forty-eight out of the forty-nine genera of the family. Her systematic results are very instructive: not only did she find it possible to draw up a key for the identification of the genera, but in the three genera (with six to twelve species) in which a fairly complete specific survey was made, as well as in several smaller genera, the spodograms were also found to be distinctive in each species. Generically the spodograms were found to group themselves in a way which corresponded on the whole with the established subfamilies.

Attempts have naturally been made to find cuticular distinctions between closely similar species or varieties in critical genera, but, as perhaps might be expected, the distinctions in such cases often seem to be slight or unrecognisable. Thus Sawyer (1932) found no taxonomic differences in the stomata of four cultivated varieties of *Vaccinium macrocarpon*, nor could he distinguish these from *V. corymbosum*. Incidentally he gives the stomatal frequency in these plants as 632 per sq. mm., and states that this is the highest number ever recorded for any plant, but much higher frequencies occur, for example, in the Myrtaceae—see below—and stomatal frequencies of six or seven hundred are probably not uncommon. Weiss (1865) recorded a frequency of 1072 per sq. mm. in *young* leaves of *Olea europaea* (625 in mature leaves), and quoted Unger's figures for *Brassica rapa* of 717 in the lower epidermis together with 374 in the upper epidermis, but obtained frequencies above 600 in only 2 per cent. of the *mature* leaves which he examined. The highest recorded frequency known to me is 2200 per sq. mm. in *Veronica cookiana* (Adamson, 1912, p. 268); this must be very exceptional.

Bancroft (1934, 1934 a) has investigated the number of stomata per unit area in certain species of *Salix* (in which they can be distinguished as "surface dots" with a lens), and though she has little faith in the value of this character among the willows, she thinks that if completely comparable material could be used, rough estimates of stomatal frequency even as made with a lens might be of some help in diagnosis. Thus *Salix alba* was found to have about twice as many stomata per sq. mm. as *S. fragilis*. On the other hand, in *S. alba* and *S. alba* var. *caerulea* the number was approximately the same; this result is, however, scarcely surprising when we learn that one of the greatest living authorities on *Salix*, Dr Floderus of Stockholm, "is unable" (presumably with the whole plant before him) "to recognise var. *caerulea* as distinct from *S. alba*".

The question of stomatal frequency as a taxonomic character has also been investigated by Baranov (1924). He admits not only that the number of stomata is inconstant in leaves from different stages of the plant, but that it varies from base to summit of one and the same leaf. However, he finds that the stomatal number in the median region of a leaf from the middle stage of the plant agrees closely with the *average* stomatal number calculated from various parts of leaves of all stages. Thus in a species of *Acantholimon* (Plumbaginaceae) the median region of the two sides of "central" leaves gave

Lower epidermis	59·6 per sq. mm.
Upper epidermis	80·1 per sq. mm.

while the average calculated from various parts of leaves from all parts of the plant gave

Lower epidermis	59·63 per sq. mm.
Upper epidermis	80·98 per sq. mm.

In studying stomatal frequency as a specific or generic constant in recent plants, either the average for the whole plant or (as Weiss had already laid down in 1865)

the median portion of an average leaf should therefore always be taken, but of course the method cannot be applied in full to fossil plants.

Loftfield (1921, p. 73) noted briefly that in leaves of *Malva rotundifolia* with varying stomatal frequency the ratio of stomata to the epidermal cells was the same, and this aspect of the question has been carried further by Salisbury (1927), whose work on various plants suggests that this ratio may be to some extent a specific constant. Thus in *Sambucus nigra* "the difference in stomatal frequency between the sun-leaves and shade-leaves is almost entirely accounted for by the extent of growth of the epidermal cells and not to differences in the proportion of stomata produced" (p. 53), and this is also true for variations in frequency in different parts of the same leaf, and in leaves from plants grown on dry and wet soils. Since then "under a given set of conditions a species tends to form a definite proportion of stomatal initials", Salisbury proposes to establish a *Stomatal Index* to express the percentage of stomata in the total number of epidermal cells per unit area (p. 50). This stomatal index would seem to give a more precise value to stomatal number considered as a taxonomic character than frequency alone, or than the average frequency suggested by Baranov.

Brief reference may be made to another variable character: average length of stomata. Odell (1932, p. 954), to prove that this character is "of no use in the determination of Angiosperms", quotes two species of *Ficus* which both have an average stomatal length of 0·017 mm.: "*Ficus vogelii*, however, has a range of lengths from 0·012 to 0·025 mm., whereas *Ficus lyrata* has a constant length of 0·017 mm." Obviously if the figures are correct this character would at once serve to distinguish the two species in question.

Among the systematic studies of epidermal structure in certain families (in addition to those summarised by Solereder) may be mentioned Krafft's work on the Menispermaceae (1907) and Grosse (1908) on the Myrsinaceae. In more recent years Bandulska has described living members of the Fagaceae (1924), Lauraceae (1926, 1928), and Myrtaceae (1931), as a preliminary to the identification of fossil Eocene cuticles; these are not complete surveys of the families in question, and deal in detail only with those genera which have also been recognised in the Eocene beds of Bournemouth. However, in the Lauraceae, for example, this author after an examination of twenty-three recent genera finds a very distinct family resemblance in stomatal structure. Moreover, "in studying the recent cuticles, a casual inspection impresses one with the differences among the species of a genus; nevertheless, when attention is concentrated on the stomatal apparatus, striking similarities become manifest between recent species of the same genus and between fossil and recent forms" (1926, p. 384). *Aniba* is a good example of a genus with a distinctive cuticle; the stomata, confined to the lower surface, have "depressed guard cells with scales, bordered by two or four accessory cells, which in some species are markedly lobed and overarch the pore to which they impart a cruciform poral outline. The free edge of the accessory cells is strongly thickened in various species of this genus. Spicular ridges may be present in guard cells, accessory cells, and parenchyma" (Bandulska, 1926, p. 420). On the other hand, the two closely allied

genera *Litsea* and *Neolitsea* do not seem to show any clear or constant anatomical distinction in their cuticles, except that the cell walls of the latter are usually more markedly sinuate.

In the Myrtaceae the occasional occurrence of relatively large stomata among numerous small ones may be noted, also the collar-like hair bases and the girdle of cells encircling the guard cells. In *Tristania* these girdling cells are particularly distinctive, and the stomata are exceedingly numerous. Bandulska does not give actual figures of frequency, but a few counts from her cuticle preparations of *T. suaveolens* in the British Museum gave from 1000 to 1100 stomata per sq. mm. This is very much greater than the number noted by Sawyer (1932) in *Vaccinium macrocarpon* as probably the highest recorded frequency, and several other species of *Tristania* ranged from 700 to 900. In various species of *Rhodomyrtus* the stomatal frequency is from 500 to 700 per sq. mm.; in *Myrtus* and *Eucalyptus* the numbers are not so high.

Clearly even rough estimates of stomatal frequency in fragments of cuticle may often be of value, especially of course when taken in conjunction with other characters. There is a considerable difference between the *Salix fragilis* recorded by Bancroft (1934) with only 50 stomata per sq. mm. and a *Tristania* with about a thousand, but everyone would agree with Bancroft that such characters "do not provide consistent and reliable diagnostic data unless used with extreme discretion". One would expect from analogy that stomatal frequency might in some groups be a family character, in others generic, in others specific, and doubtless in many cases of no systematic value at all when considered separately. Short of a complete survey, which is practically out of the question, every case must be considered on its merits. Large genera are more likely to have wide variations in stomatal frequency than small ones; thus *Ficus elastica* has from 120 to 150 large stomata per sq. mm. whereas *Ficus infectoria* has from six to seven hundred small ones. (Cf. Satake's remarks on the spodograms of *Ficus* quoted above.)

V. FOSSIL DICOTYLEDONS.

The earliest figures of fossil dicotyledonous cuticles which have come under my notice are those given by Goeppert and Berendt (1845) of Oligocene leaves in Baltic amber. They illustrate three species of *Dermatophyllites* (referred to Ericaceae, but later regarded as dubious by Goeppert himself and by Conwentz), and an *Alnites*, as well as an unnamed cuticle without stomata, but the figures are too diagrammatic to be of any value. The possibilities of the cuticular study of amber plants do not seem to have been explored.

Schleiden's figures (in Schmid and Schleiden, 1846) of minute fragments of epidermis (named *Phyllites ungerianus*) obtained by macerating brown coal may be ignored as valid records of dicotyledons; the age is uncertain and the figures untrustworthy.

Brodie (1842, p. 592) in a note on the Eocene plants of Bournemouth remarked that "when the sandstone is freshly broken the epidermis of the fossil frequently

peels off", but he does not seem to have examined it any further, and one of the first to make a microscopical study of such fossil cuticles as an aid to identification was Weber, who records (Wessel and Weber, 1855, p. 113) that in the winter of 1852 he examined leaves from the Brown Coal of Orsberg and Rott near Bonn, in which a transparent epidermis showed under the microscope the form and arrangement of stomata and epidermal cells. Having named his leaves from their external appearance, he proceeded to compare the cuticles with those of living species, and found that the structure confirmed the identifications. "Ein wahrer Triumph der Paläontologie!" he exclaims, but in spite of this announcement of a valuable new weapon of research, it was not applied to the study of fossil dicotyledons for another half century. The cuticles which Weber figures are named *Acer pseudocampestre*, *Ceanothus zizyphoides*, *Juglans deformis*, *Prunus prinoides*, and *Sambucus celtifolia*; the drawings are rather diagrammatic and the descriptions brief. A re-examination of these cuticles would be of great interest.

Bornemann (1856) who produced the first important work on fossil cuticles (Triassic cycads) discussed the question of their value at greater length than Weber, and dealt incidentally with the cuticles of some recent flowering plants. Bunbury too suggested (1859, p. 52) that "in those rare cases where the fossil leaves are so well preserved that the cellular structure of the epidermis, and its pores or stomata, can be satisfactorily examined, these may probably afford valuable aid towards the determination of affinities". He went on to urge a comparative study of these characters in recent plants, and stated as an instance that the structure of the stomata in *Ginkgo* was "exceptional in the family of Coniferae". It is interesting to note that though *Ginkgo* was then included in the conifers, its isolated position had been foreshadowed by an examination of its stomatal structure. Even the gymnosperms, however, whose cuticles are so frequently preserved in the fossil state, were largely neglected for many years after this.

Unger (1852) had slightly preceded Weber in figuring a leaf cuticle, from the Miocene of Styria, which he named *Potamogeton morloti*. He did not realise that a thin-leaved water plant would be unlikely to produce a thick and resistant fossil cuticle, and his comparison with the cuticle of a recent *Potamogeton* with floating leaves was not very exact. Nor did he notice that the upper and lower epidermis of his fossil were identical in structure and both bore stomata. After a lapse of more than half a century, Knoll (1904) re-examined these leaves and, by a detailed study of their venation and cuticular structure, was able to show that they were members of the Loranthaceae; he renamed them *Viscophyllum morloti*, for while in some characters they were very close to the living *Viscum*, in others they were nearer to *Phoradendron*.

The cuticle of another species of *Viscophyllum*, *V. miqueli* from the Upper Pliocene of the Klärbecken near Frankfurt, was figured by Engelhardt and Kinkelin (1908, p. 246, Pl. XXXII, figs. 7, 8); this leaf also had originally been described as a *Potamogeton* (Geyler and Kinkelin, 1887, p. 20). Kräusel has described the same species and figured its cuticle from the Miocene of Schossnitz in Silesia (1921, p. 428, Pl. X, figs. 3, 4 [unnamed]; 1929, p. 33). Engelhardt and Kinkelin (1908)

also figure, somewhat diagrammatically, the cuticles of *Buxus sempervirens* var. *fossilis* (Pl. XXXIII, fig. 2) and *Ilex aquifolium* var. *fossilis* (Pl. XXXIII, fig. 4).

Colani (1920) figured a few fragments of dicotyledonous cuticles from the Tertiary of Indo-China, but they do not seem to be very well preserved. Stomata are neither figured nor described, and most of the fragments are from the upper epidermis. The imperfect leaves from which they were obtained were doubtfully referred to such genera as *Laurus, Cinnamomum*, and *Ficus*, and on some of them are epiphytic fungi.[1]

Johnson and Gilmore (1921) describe the cuticle of some leaves from Irish Tertiary beds which they refer to *Dewalquea* Saporta. They name three new species of this supposed extinct genus; the figures of the epidermis on Pl. XII are apparently all of *D. hibernica* J. and G., though this is not stated. External form of the leaf as well as epidermal structure (including the presence of peltate hairs) are said to suggest an affinity either with Juglandaceae or with Oleaceae, but the authors do not seem to have made a really detailed examination and comparison of the cuticles of these families, which is precisely what is required before one can arrive at any conclusion. Possibly more light will be thrown on the matter in a paper on *Dewalquea* by Johnson, read before the Geological Society in 1933 but not yet published. The identification of the Lough Neagh fossil leaves with *Dewalquea* was made on the basis of external form alone.

The cuticle of *D. gelindenensis* Sap. from the Lower Eocene (Landenian) of Belgium has recently been described by Stockmans (1932); the apparent absence of peltate scales and cuticular striae suggests that *D. gelindenensis* and *D. hibernica* may not belong to the same genus. The preservation of the Belgian cuticle is imperfect, and the published figures of the Irish cuticles are not very clear (partly perhaps owing to poor preservation), so that an exact comparison is not easy. Stockmans does not discuss the affinities of his specimens.

Kräusel (1922, p. 25), in studying Cretaceous dicotyledons from borings in Holland, found that as a general rule small fragments of leaf membranes did not show any special diagnostic characters. He obtained a species of *Myrica*, however, which agreed very closely both in leaf-form and epidermal structure with a living species of the genus. In both *M. pseudoquercifolia* Kräusel and in *M. quercifolia* L. from South Africa the epidermis consisted of small polygonal straight-walled epidermal cells with small slightly sunken circular stomata (in the lower epidermis only) surrounded by a ring of subsidiary cells, and the same type of spherical druses occurred in both.

In a series of papers published since 1923 (see References) Bandulska has been investigating the Eocene (Lutetian) flora of Bournemouth—an abundant assemblage of leaf impressions some few of which still retain their cuticles, as Brodie noticed

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[1] The bodies found by Colani (1920, pp. 441–5) on an unidentified dicotyledonous leaf from the Tertiary (Mio-Pliocene?) of Dong-Giao (Indo-China) are not multicellular hairs but fungi belonging to the Microthyriaceae. Her Fig. 43 seems to represent a thyriothecium with a definite ostiole, while Fig. 47 is probably a stigmocyst. On Pl. XXIX, fig. 7, she shows a fragment of cuticle with apparently three thyriothecia (Fig. 43 represents the largest of these much magnified) and numerous hyphae, but it is impossible, at any rate from the figures and description, to determine the nearer affinities of this fungus.

nearly a hundred years ago. Although many of the cuticles remain at present unidentified, Bandulska has been able, by an intensive cuticular study of certain recent families whose presence in the Eocene was indicated on various grounds, to prove the existence in the Bournemouth beds of several living genera. Her work on the Lauraceae (1926, 1928, 1929) is particularly noteworthy, and has already been discussed above; the fossil genera identified are *Aniba*, *Neolitsea*, *Litsea*, *Lindera*, and *Cinnamomum*. Three species of *Litsea* are differentiated, and two or three of *Lindera*, on characters such as the absence or presence and relative abundance of hairs, thickness of cell walls, sinuosity or straightness of cell walls, stomatal frequency, and so on; characters which also differ among the living species. In no case was a fossil cuticle found to be indistinguishable from that of a living species, and indeed evidence from leaves alone, even when the anatomy is preserved, would not warrant the identification of an Eocene with a recent species. Some of the Bournemouth cuticles are quite unmistakable—*Aniba spiculata*, for example, which is very abundant; it is even possible, when one becomes familiar with the material, to recognise small fragments of the cuticle of this species before maceration from the colour and texture alone. Examination of Bandulska's preparations, now in the Geological Department of the British Museum, confirms the impression that careful and well-illustrated work of this type is likely to yield results of great value, and in the main there seems no reason to dissent from her identifications, though there may be some difference of opinion on some of her specific or varietal distinctions—in the case of fossils which are mere fragmentary parts of a plant the establishment of varietal names seems an unnecessary complication of the nomenclature—or on the line to be drawn between two very closely allied genera. Miss B. Benzian has, however, pointed out to me that there is a strong possibility of confusion between the two leaves described by Bandulska as *Fagus bournensis* (1924) and *Rhodomyrtus sinuata* (1931). Further material will probably be required in order to elucidate this point. *Folium rosiforme* Hofmann (1932, p. 63; 1934, p. 214) from the Eocene of Geiseltal is perhaps identical with *Rhodomyrtus sinuata*. Another member of the Myrtaceae, *Tristania bournensis*, has been described by Bandulska, who has also recognised this genus in the Pliocene of Tuscany. The latter identification (*Tristania toscana*) is of special interest because the specimen from which the cuticle was obtained has been labelled in manuscript as a *Persea*; microscopic examination, however, showed at once that the cuticle was quite unlike that of a *Persea* or any other lauraceous genus.

Reid and Chandler (1926) obtained a few leaves with cuticle preserved among the Bembridge Limestone fruits and seeds; *Neolitsea* was identified by the aid of its cuticular structure, and two species of *Cinnamomum* were figured and described. They also figured the cuticles of two fragmentary and unidentified leaves. Stockmans (1932) has dealt with the cuticle of *Litsea elatinervis* from the Eocene of Belgium, and states that it closely resembles the species described by Bandulska. Hofmann (1932) has figured the cuticles of two species of *Cinnamomum* from the Eocene of Geiseltal, which also seem to agree in general with those described by Bandulska (1928).

In a paper on Lower Miocene plants from Styria Hofmann (1926) describes and figures (rather too diagrammatically to be of much value to other workers) cuticles of leaves referred to *Quercus, Castanea, Ficus, Sapindus, Ilex, Symplocos, Bumelia, Plumeria, Ligustrum,* and *Viburnum.* Stomata are not always figured and the leaves are in all cases compared with living species, but there is not sufficient detail for a really accurate comparison. The Eocene brown-coal of the Geiseltal yields well-preserved dicotyledonous cuticles; in 1930 Hofmann figured a few of these (*Myrica,* cf. *aethiopica*—a living species—and *Celastrus oxyphyllus* Unger are named) but there are no detailed descriptions. In a later paper (1932) Hofmann gives up her scarcely defensible course of equating early Tertiary cuticles with those of living species, and, going to the opposite extreme, adds a new terror to palaeobotanical nomenclature by proposing a provisional genus for unidentified cuticles. Thirteen species of this new "genus" *Folium* are figured; some of the photographs are excellent, but the descriptions are again inadequate. Some of the new "species" are scraps of upper epidermis only, without stomata, and it may well be doubted whether such scraps are worth figuring at all, let alone being dignified with a name. In this paper and in another brief note (1932 *a*) Hofmann seems to think that it is more important to publish provisional names for un-identifiable fossil cuticles, since so little is known of recent forms, than to settle down to the really fundamental work of studying both in detail. The name *Folium* is really superfluous, since *Dicotylophyllum* is already in use for fossil dicotyledonous leaves of uncertain affinity. Hofmann's *Paläohistologie der Pflanze* (1934) devotes a section to fossil angiosperm cuticles.

Florin (1926) showed by a study of the cuticle that certain leaves from the Oligocene of Flörsheim (Main basin) which had been described as *Podocarpus* by Engelhardt were in fact not conifers at all but dicotyledons. In this particular case, although he examined the cuticles of numerous recent *Podocarpus*-like dicotyledons, he was unable to identify the fossil exactly and named it *Dicotylo-phyllum neglectum.*

Upper Pliocene plants from the Harz region have been described by Straus (1930). In comparatively recent geological deposits leaf cuticles are perhaps more frequently preserved than in the Upper Cretaceous and early Tertiary, and since the flora approaches that of the present day the field of comparison is fortunately restricted. Cuticular structure is here a valuable adjunct, especially perhaps in confirming the identification of genera. Straus figures and describes several cuticles (many of them with good photographs of high magnification), including some which are identified on various grounds as belonging to living species (e.g. *Populus monilifera, Hedera helix, Aesculus hippocastanum*), but in other cases the cuticles of living species are either insufficiently known or do not exhibit any appreciable distinctions. Thus Straus identifies certain leaves as *Juglans sieboldiana* Max. var. *fossilis* Nath.; the epidermal structure supports the generic identification, but the leaves of *J. nigra, J. cinerea,* and *J. cordiformis* are also apparently very similar in external form as well as minute anatomy. On the other hand the cuticle of a *Populus* compared with *P. tremula* does differ slightly from that referred to

P. monilifera. Other cuticles figured by Straus (some of them only fragments of the upper epidermis without stomata) include species of *Salix, Zelkova, Sassafras, Acer,* and *Crataegus*. In discussing the identification of cuticles Straus remarks that works like Gilg and Brandt's *Lehrbuch der Pharmakognosie* often contain useful illustrations of epidermal structures.

Berry (1933) describes and figures the cuticle of an Eocene leaf from Mississippi which he identifies (from its external appearance) as *Combretum petraflumense,* but he does not deal with the cuticular structure of comparable living forms.

Johnson (1933) figures (not very clearly) and briefly describes the cuticle of an Irish Tertiary species of *Platanus,* which he names *P. hibernica*; the stomata and epidermis are said to be closely similar to those of the living *Platanus orientalis.* In the same deposit he found numerous detached candelabra-hairs resembling those characteristic of living plane trees. Johnson's cuticles came from Washing Bay, Lough Neagh, whence he records two varieties of *P. hibernica*: var. *guillelmae* (Goeppert) and var. *populoides* nov., but he does not say whether their cuticles are distinguishable, nor which of them yielded the figured specimen. His type specimen of *P. hibernica* (Pl. I) is an impression only without cuticle, and does not come from Ballypalady, as he states, but from Sandy Bay, Lough Neagh.

Although this review is concerned primarily with anatomical characters of leaves, it may not be out of place to refer briefly to a recent important work on fossil fruits and seeds (Reid and Chandler, 1933) in which extensive use is made of comparable anatomical features. In general, these authors consider that "anatomical characters of fruits and seeds are among the most reliable, because among the most stable and persistent inheritances from the past" (p. 28), and they emphasise, as of most value, the mode of germination, the arrangement of the various carpellary organs, and the structure and succession of the integuments. One or two instances of the use of histological characters in fruits and seeds may be mentioned. The fruits of the Eocene Lauraceae, which are not recognisable from their external appearance alone, possess very characteristic stellate groups of cells in the mesocarp, and in the seeds of the same family one coat of the testa is almost invariably formed of large secreting cells. The authors consider that certain combinations of macroscopic and microscopic characters afford absolutely conclusive evidence of the presence of Lauraceae, though the generic position might be very difficult to ascertain. The family Euphorbiaceae is also "very clearly characterised by the features both of the fruit and seed, which are remarkably uniform and constant" (p. 283), though it proved difficult to trace generic relationship. In this family, however, minute anatomical characters, such as the size of the testa cells and the thickness of the columnar coat, were found to be *specifically* constant. Finally, one may cite from the Icacinaceae an instance in which the cellular structure of the fruit is of value in discriminating *genera*: the papillate locule-lining serves to distinguish *Iodes* from *Natsiatum*.

VI. SUMMARY.

The study of cuticular structure as an aid to identification and classification, first extensively applied to fossil plants (particularly gymnosperms), is being increasingly used in recent taxonomic researches.

The investigation of spodograms (the characteristic figures due to siliceous skeletons, crystals, and the like seen in the residue of leaves slowly reduced to ash) has proved of great value in the Gramineae especially, and among dicotyledons in the Urticales. Attempts to apply the method to fossil plants have not so far led to any results.

Prat's analysis of epidermal characters in the Gramineae indicates the principles on which detailed taxonomic work should be based, and the systematic results he has so far obtained in this family are supported by evidence from other sources. Similar surveys of dicotyledonous families have scarcely yet been attempted; Bandulska's work on the Lauraceae is especially worthy of mention.

The available evidence suggests that in cases of convergence of leaf form, structural differences in the cuticle will almost always provide a means of distinguishing isolated leaves.

The *distribution* of cuticular elements may often furnish a means of discriminating closely related taxonomic groups which cannot be differentiated by purely structural characters.

Statistical characters such as size or frequency of the elements, though often of value, must always be used with extreme caution. The ratio of stomata to epidermal cells (the stomatal index) is less variable than mere stomatal frequency.

Individual cuticular and other epidermal characters cannot be considered in isolation in taxonomic work. As with other features used in classification, the sum of the available characters must be taken as the basis.

The literature on fossil angiosperms, here summarised, is already extensive, particularly on the dicotyledons. Valuable results, however, can only be expected from full and detailed investigations of well-preserved cuticles, together with a close comparison with a wide range of living forms.

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PHOTOSYNTHESIS IN INTERMITTENT LIGHT, IN RELATION TO CURRENT FORMULATIONS OF THE PRINCIPLES OF THE PHOTO-SYNTHETIC MECHANISM

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CONTENTS.

I. INTRODUCTION.

VARIOUS workers who have accumulated data upon the assimilative activity of green plants have formulated their conceptions of the photosynthetic mechanism in so far as it concerns their particular line of experimentation. The present writer has spent some time in studying these formulations in connection with his own data and is continuing the endeavour to present a generalised formulation which takes account as far as possible of all the available data and acts as a test of the concordance of such data and an index of what further experiments are needed.

As part of this general survey there is here presented a special section on the relation of the data of Emerson and Arnold (1932, 1933) to the formulation already presented by the writer (Briggs, 1933).

The present state of our knowledge of photosynthesis in green plants as represented by the results of experimental investigations is extensive, but considered from the aspect of the correlation of these data our knowledge is far from profound. As is the case with most reactions proceeding within the cell our information with regard to the conditions at the seat of the reaction is much less precise than when the reaction is *in vitro*. Complications arise partly from the difficulty of ascertaining the difference between the intensity of such factors as concentration of carbon dioxide and intensity of illumination outside the cell and the places where the reaction is going on inside the cell, and partly from the fact that other metabolic reactions in progress may affect the concentration of substances involved in or otherwise determining the rate of the reaction under consideration. With the assimilatory reduction of carbon dioxide there is the ever-present complication of the respiratory oxidation of carbohydrates to carbon dioxide. Apart from any possible linkage of these two processes (Briggs, 1933), experiment can tell us only the difference between their opposing activities.

Although as indicated, and as will appear more clearly as we proceed, any attempt at an interpretation of the great variety of data is beset with many difficulties, yet we hope that our attempt will show that some insight has been gained. There is no shortage of suggestions as to the mechanism of photosynthesis, but so far no detailed consideration has been given to the survey of how far the schemata are capable of explaining the wide range of experimental data available. We propose to set out such a detailed consideration elsewhere, and in the present article to restrict ourselves mainly to an attempt to interpret the results of experiments, mostly recent, on assimilation in intermittent illumination. We shall give only a brief consideration of the results of the far more extensive investigations in continuous illumination. With this limitation we may appear to ignore some obvious difficulties and to suggest a finality for our interpretation which it does not really possess. Of many of the difficulties we are aware, and that our suggestions are tentative we fully recognise. So many suggestions have already been and are being made as to the nature of the mechanism that it is impossible to give an account of them here or to point out in what respects the present interpretation agrees or disagrees with each of these.

II. THE RELATION BETWEEN THE INTENSITY OF VARIOUS FACTORS AND ASSIMILATION IN CONTINUOUS LIGHT AND SUGGESTIONS AS TO THE MECHANISM OF PHOTOSYNTHESIS.

(1) *Mechanism.*

The first fact which stands out is the general lack of proportionality between the rate of assimilation and the amount of chlorophyll present which has been so clearly demonstrated by Willstätter and Stoll (1918). There is the additional fact that even when illumination is weak the assimilation of young leaves increases with

age even when the chlorophyll is maintained at a constant value (Briggs, 1920). At various times the suggestion has been made that chlorophyll combines with carbon dioxide, is then activated by light energy and subsequently broken down into free chlorophyll and products. This suggestion has been revived recently (Baly, 1934; Emerson and Green, 1934). As formulated the schemata imply that when the concentration of carbon dioxide and intensity of illumination are very great the whole of the chlorophyll will be in the form of the activated compound and that the rate of the reaction will be proportional to this and hence to the amount of the chlorophyll present. Admittedly Baly (1934) suggests that the rate is also proportional to the concentration of some other substance present. If variation in this other substance is to be accepted as the explanation of the variation in the rate of assimilation per unit amount of chlorophyll (assimilation number of Willstätter and Stoll (1918)), then we should have to assume that this factor decreased as leaves developed and increased in chlorophyll content, and that leaves of green varieties had less than had leaves of yellow varieties. As we shall see later Emerson and Arnold (1933) claim that when *Chlorella* cells are illuminated by a very intense flash of very short duration the maximal amount of carbon dioxide reduced per flash is of the order of as little as one molecule per two thousand of chlorophyll present. If each molecule of chlorophyll were combined with one molecule of carbon dioxide and they were all activated by the flash we should expect a molecule of carbon dioxide to be reduced for every molecule of chlorophyll. The suggestion that only a small fraction of the chlorophyll is effective will not in itself account for the fact that the form of the relation between assimilation and intensity of illumination depends upon the amount of chlorophyll.

The above-mentioned schemata also imply a relation between rate, R, and concentration of carbon dioxide, C, or intensity of illumination, I, of the following type:

$$R = \frac{k_1 C}{C + k_c},$$

$$R = \frac{k_2 I}{I + k_i}.$$

These expressions indicate that the relation between $1/R$ and $1/C$ and that between $1/R$ and $1/I$ are represented by straight lines. Graphs of $1/R$ against $1/C$ and of $1/R$ against $1/I$ are a ready means of testing the agreement of the data with such a schema, and the values of $-1/C$ and $-1/I$ when $1/R$ is zero indicate the values of $1/k_c$ and $1/k_i$ respectively.

The schema we have already suggested (Briggs, 1933) accounts for the non-proportionality of rate to amount of chlorophyll and implies a non-linear form for the above relations. It is as follows. We assume that carbon dioxide combines in a reversible manner with a substance S to give a complex S_c. As to whether S is identical with chlorophyll or not we cannot say from the evidence at present available. This complex is activated by the absorption of light energy and so converted to S_l. We make no suggestion as to the nature of this substance—it may be a peroxide as suggested by Willstätter and Stoll (1918), but for our present purpose its precise nature has little significance. Finally we assume that S_l is broken down with

the aid of a catalyst B to give the first carbohydrate product of photosynthesis, oxygen and free S substance. Moreover, we suggest that the decomposition of S_l is by way of an intermediate compound of S_l and B, namely X.

$$S + CO_2 \rightleftharpoons S_c,$$
$$S_c + \text{energy} \rightleftharpoons S_l,$$
$$S_l + B \rightleftharpoons X,$$
$$X \rightarrow B + S + \text{products.}$$
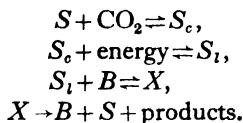
As the result of long consideration of the data available we think that no simpler schema will suffice; on the other hand, it may have to be elaborated as new facts accumulate.

The same schema has been used more recently by James (1934), with the exception that he has assumed that the rate of breakdown of S_l is proportional to $B . S_l/(S_l + K)$. This assumption is only justifiable for enzyme reactions so long as the fraction of S_l in the form X is negligibly small. Clearly this becomes less justifiable the smaller S_l is made. The only aspect of the schema which he considers is that where the intensity of the illumination is very weak, that is where S_l is small.

If the S substance was chlorophyll then our schema would be like that suggested by Willstätter and Stoll (1918). Their formulation is precise as to the nature of the linkage of the CO_2 to the chlorophyll and the change it undergoes on activation, but they make no formulations of a kinetic nature. Another schema which would explain the non-proportionality of rate to chlorophyll and give a similar non-linear relation between $1/R$ and $1/C$ and between $1/R$ and $1/I$ is as follows and can be made a modification of the first schema. The chlorophyll is activated by absorption of radiant energy which is readily handed on to carbon dioxide which is bound to a substance S, thus converting this to an active state, S_l. This activated compound is broken down catalytically by B to give the products.

$$\text{Chl} + \text{energy} \rightleftharpoons \text{Chl}',$$
$$S + CO_2 \rightleftharpoons S_c,$$
$$S_c + \text{Chl}' \rightarrow S_l + \text{Chl},$$
$$S_l + B \rightarrow S + B + \text{products.}$$
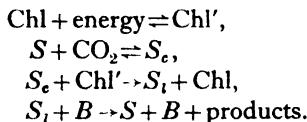
If the interaction of S_l and B gave an intermediate product, X, before breaking down then this schema differs from the previous formulation only in that the activation of S_c is photosensitised by chlorophyll rather than directly by light.

If we had assumed that the products arose by the direct interaction of activated chlorophyll and S_c, the schema would be in essence that suggested by Warburg (1920). Later he abandoned this early schema because he considered that there was evidence that the process involved the breakdown of a peroxide (Warburg and Uyesugi, 1924). Although he made no definite formulation of his modified views, if S_l in the above schema were a peroxide then the schema would incorporate the earlier views and the later modification.

The detailed mathematical formulation of the consequences of the above schema would occupy far too much space here, but we can indicate shortly how the various experimental results with continuous illumination can be interpreted. Our premises

are clearly stated, and it is only a matter of straightforward mathematical argument
to arrive at the conclusions we give.

(2) *Amount of chlorophyll.*

Clearly our schema accounts for the fact that, when the concentration of carbon
dioxide and intensity of illumination are great, the rate of assimilation is not pro-
portional to the amount of chlorophyll. It increases less rapidly than the amount of
S, and if this is great enough approaches the limit of proportionality to B when the
latter is saturated with S_1. Further, difference in chlorophyll content has a greater
effect on the rate at low intensities of illumination than at high. This is in accord
with the results of Willstätter and Stoll (1918).

(3) *Intensity of illumination.*

The relation between $1/R$ and $1/C$ and that between $1/R$ and $1/I$ are expressed by
curves with increasing slope as $1/R$ increases. We could, if necessary, produce the
results of many experiments showing relations of the predicted type, but in view of
the many complexities, some of which are indicated below, little could be deduced
from the agreement without prolonged consideration which would be out of place
at present. In the first place our statement about the relation for I applies to a
system where the intensity of illumination is the same at all points. This is clearly an
ideal that is never achieved in practice. When stationary material such as leaves or
shoots are used the intensity of illumination at any one absorbing centre will remain
sensibly constant, but with an agitated suspension of unicellular algal cells such as
has been used in many of the recent investigations the intensity will be constantly
changing. In the latter case, if the change of illumination of each centre is suffi-
ciently rapid our knowledge of assimilation in intermittent illumination would
suggest that the system would behave like one with uniform illumination throughout
equal to that of the mean illumination throughout time which each absorbing centre
experiences. The relation between rate and intensity of illumination would be the
same as that for our ideal system except that some fraction of the incident illumina-
tion would have to be used instead of the value itself; there would be no alteration in
the form of the curve relating $1/R$ to $1/I$. For the system with stationary centres the
assimilation for a given intensity of illumination incident upon the system is not the
assimilation for the mean of the illumination at the various absorbing centres but
the mean of the assimilation for the various intensities of illumination to which the
different centres are exposed. Our knowledge of the optical properties of the assimi-
latory systems is too limited to justify our attempting to add terms to our equations
to allow for this complication, it is sufficient to note that this extra factor makes the
slope of the curve of $1/R$ against $1/I$ (I being the incident illumination) steeper as $1/R$
becomes smaller, that is, makes the relation approach more closely to linearity. It is
impossible to say how closely the agitated suspensions of algal cells used by various
investigators approach to that very rapid agitation which would make them com-
parable to a system with uniform illumination throughout. There is a further point
worthy of notice. The weakest illumination in any of the systems used, whether leaf

or suspension of algal cells, is not necessarily as great as that emerging from the side remote from the source of illumination. Emerson and Arnold (1933) have calculated that a concentration of 10 mg. of chlorophyll per litre requires a thickness of 2·1 cm. to reduce the intensity of light of wave-length 6600 Å. to one-tenth. The average concentration of chlorophyll in *Chlorella* cells is as high as 15000 mg. per litre. An individual cell of 3 μ in diameter would have its weakest illumination 64 per cent. of the brightest, if it acted like a homogeneous fluid. This would be greater for those wave-lengths less strongly absorbed. The concentration of chlorophyll in the chloroplasts of some leaves is considerably greater than that in *Chlorella* cells. The percentage of light transmitted by a suspension of cells can be made to approach a hundred by reducing the density of the suspension without altering the illumination of the various centres within a cell, since the suspensions used are so dilute that there is very little shading of one cell by another.

The second complication is that the concentration of carbon dioxide at the assimilatory centres tends to be less than that in the medium supplying this reactant. This applies even when the CO_2 is supplied as bicarbonate ions, for here the hydrogen ions used up in combining with the bicarbonate ions will be in lower concentration at the centres than outside the cell. The difference between effective and external concentration, the only concentration which we can measure, increases as the intensity of illumination and hence the rate of assimilation rises. This factor will make the slope of the curve of $1/R$ against $1/I$ fall off more rapidly as $1/R$ falls. There is also the effect of respiration on the concentration of carbon dioxide.

(4) *Concentration of carbon dioxide.*

The same complexities disturb the relation between the rate and the concentration of carbon dioxide. This relation depends upon the intensity of illumination, and hence the complication for a system with non-uniform illumination. If we assumed a simple form for the relation between rate and concentration at the assimilatory centres, then taking the rate of supply to be proportional to the difference between this concentration and that in the external medium we could develop an expression for the relation between the rate and the external concentration. This James (1934) has done and then compared the calculated values of the rate with those observed in experiments by Van den Honert (1930) and by himself (James, 1928). A consideration of the results of such a procedure reveals its limitations as long as we have no better data than those at present available. With the former set of data there is only a limited range of rates, 31–59, and even then the difference between the calculated and observed values exceeds 10 per cent. in places. There is a greater range of rates in the other set of data, but here the difference in one case exceeds 20 per cent. Although this lies within the limits of experimental error we think that the conclusion to be drawn is not that "the theory may be considered to give a close numerical prediction of the facts", but that the experimental error is so big that numerical comparisons are not worth while with such data. It has been necessary to take this point in some detail to justify our present procedure.

(5) Temperature.

When the concentration of carbon dioxide is very great, that is, when a further increase has no appreciable effect, and the intensity of illumination very small, then the rate tends to be limited by the absorption of light energy only, and hence the recorded negligible effect of change of temperature (Warburg, 1919) under these conditions is to be expected. When the intensity is high the effect of temperature depends upon whether or not practically the whole of B is combined in the form X over the whole range of temperature. If it is, then the temperature coefficient of the rate of assimilation is the same as that of the rate of breakdown of X. But if with increase of temperature there is a decrease of the amount of B combined then the temperature coefficient will be smaller. The marked fall of the temperature coefficient with rise of temperature, which is well established, may well be explained in this way. Here also may lie the explanation of the fact recorded by Willstätter and Stoll (1918) that the temperature coefficient is smaller for leaves of yellow varieties than for those of green. The former may not have enough of the S substance to maintain the whole of the B substance in the combined state at the higher temperature.

When the concentration of the carbon dioxide is very small then the chief factor determining the rate of assimilation is the rate at which the carbon dioxide can combine with the S substance, or if it is some special form of carbon dioxide, the hydrated or dehydrated, which is active then the rate of activation may set the pace. There are indications that the temperature coefficient is different at low concentrations from what it is at high concentrations. This is what might be expected, since different processes are tending to dominate the situation with the different concentrations. It must be noted that estimations of the temperature coefficient of photosynthesis at low concentrations of carbon dioxide are of doubtful significance. Warburg (1919) reports only the value of the sum of the net oxygen production in the light and the oxygen consumption in the dark, but it is clear from his results in general that the net oxygen production is most probably a very small fraction of the calculated value for assimilation and perhaps negative. It is unfortunate that no data are given for the temperature coefficient of respiration. The effect of increase of temperature on the rate of assimilation is the same as that on the calculated rate only if the concentration of the reactant, carbon dioxide or whatever it may be, is independent of temperature. At low concentrations of carbon dioxide change of concentration has a marked effect on the rate. Warburg (1919) makes an allowance for the effect of the change of temperature on the assumption that the concentration of carbon dioxide available is that in equilibrium with the external solution at each temperature and that the rate is proportional to this concentration. But increase in the rate of respiration with increase in temperature may well raise the concentration of carbon dioxide or other substances capable of being reduced in the photosynthetic process. There are various possibilities which we cannot discuss now; it is sufficient to have pointed out the doubtful significance of the reported values of the

temperature coefficient at low concentrations of carbon dioxide. As we shall see later there are other grounds for doubting the high values recorded.

A full consideration of the expression relating rate to temperature would show that the above presentation is a simplification. It suffices, however, for our present purposes.

(6) *Depressants.*

We turn now to a brief consideration of the effect of some depressants on the rate of assimilation. The effect of HCN is on the whole quite distinct from that of phenyl urethane. Warburg (1920) and Van der Paauw (1932) have shown that with high carbon dioxide HCN causes a greater percentage depression at high illumination than at low. That is, addition of HCN acts like a decrease of temperature. This suggests that this depressant alters either the amount of B substance or the rate of breakdown of the compound of B with S, that is X (cf. p. 463). If this is so we should expect a greater depressant effect at high concentrations of carbon dioxide than at low. Wager, in experiments as yet unpublished, found that such is the case.

Although Warburg does not draw this conclusion his results (1920) show that the depressant effect of phenyl urethane is greater with high carbon dioxide than with low—some faults in his arithmetic diminish the difference in the effect. Since his results (Warburg, 1919) show that the depressant effect is greater with low illumination than with high the urethane must act in a different manner from HCN. The effect of urethane is the same as that of decreasing illumination, that is as regards the differential effect at high and low illumination and at high and low carbon dioxide. This depressant may well act by decreasing the efficiency of the photosensitising mechanism by decreasing the amount of sensitiser or by increasing the probability of the sensitiser losing energy to the probability of its handing the energy on to the carbon dioxide.

We may conclude as a result of this very brief discussion that our schema is of the type which is in good qualitative accord with the results available. We may point out that certain modifications to our schema would not alter the general conclusions we have so far drawn. For example, the carbon dioxide may enter into the reaction by combining with B rather than with S. A more careful consideration of well-planned experiments on the interaction of illumination and concentration of carbon dioxide might help to distinguish between these two possibilities.

III. INTERMITTENT ILLUMINATION: EXPERIMENTS OF EMERSON AND ARNOLD.

(1) *General considerations.*

Turning now to a consideration of the results of experiments with intermittent illumination we shall deal first with those of Emerson and Arnold (1932, 1933).[1] It is convenient to summarise here the suggestions they have put forward to explain

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[1] Although we cannot agree with many of the conclusions drawn by the authors yet we fully realise that the results of their experiments form a very valuable contribution to our knowledge of photosynthesis.

their results. In their first paper the schema is put forward in a purely qualitative form—chlorophyll combines with carbon dioxide, this is activated by light energy (the photochemical reaction) and then breaks down to give free chlorophyll and products (the Blackman reaction as Warburg named it). As pointed out earlier there is nothing new in this for students of photosynthesis. In the second paper they make some definite suggestions as to the kinetics, namely that the photochemical reaction proceeds at a rate proportional to the amount of compound of chlorophyll and carbon dioxide unactivated and to the intensity of illumination. Finally, Arnold (1933) claims to have produced evidence that the Blackman reaction is of the first order, but his ideas of the whole reaction are so vague that they cannot be put precisely here and reference must be made to the original.

The experimental procedure was as follows. The material, a suspension of *Chlorella* cells in a solution of K_2CO_3 and $KHCO_3$, was illuminated intermittently by flashes from a neon tube. The flashes were of very short duration compared with the intervening dark periods. The former were of the order 10^{-5} sec. and the latter one-hundredth to one-tenth of a second adjusted to a constant value for each experiment. The period of the flash was so short that it seems reasonable on any theory so far suggested to assume that the assimilation during the flash can be neglected.

It must be emphasised that all values for assimilation are based on the assumption that an allowance for the respiratory consumption of oxygen in illuminated cells can be made on the basis that it is the same as that in cells in the dark. With high rates of assimilation the respiration of darkened cells is relatively small compared with the net assimilation of the illuminated cells, and hence the estimated assimilation would not be changed much if the respiration of these was taken as twice as big as that of darkened cells. But in flashing light, with long dark periods particularly, the net assimilation is very small and may even be negative, and hence the estimated value of the assimilation per flash depends largely upon the value assumed for the respiration. At present we have no line of attack on the question of respiration during assimilation and must use the estimates of assimilation, always bearing in mind the assumptions on which the estimates are made.

If the data were sufficiently accurate to justify the presentation of the solution of the equations for assimilation as a function of all the variables which the experimenters tried we should clearly have to consider the change with time of the compound of carbon dioxide with S substance, of the activated form of this compound, and of the substance X. We should have to consider the changes of these not only in the dark periods but also during the light flash. We do not think that anything is to be gained by presenting the solution of the equations, but prefer to consider the results in a more qualitative manner at the present stage.

It was found that the amount of assimilation per flash increased to a maximal value as the length of the dark period was increased, and throughout the authors treat this as a measure of the progress of the Blackman reaction. If we accept their view that the activated compound breaks down directly in this reaction then their

interpretation is correct only if the amount of this compound at the end of a flash of light is independent of the length of the dark period. Now, during the latter period the carbon dioxide compound is being regenerated and the amount at the end of the period will be a function of the duration except in the limit when the concentration of the carbon dioxide or the velocity constants of formation and breakdown of the compound are so great that the regeneration is practically complete even for the shortest duration used. Further, since the amount of the activated compound still in the activated state at the end of a dark period, that is at the beginning of the light flash, depends upon the duration of this period, the total amount at the end of the light flash will depend upon the duration of the dark period. Only in the limit as the carbon dioxide is made very great so that there is no free chlorophyll at the end of the dark period or S_c has attained an equilibrium value and the flash is sufficiently intense to activate the whole of the compound unactivated at the beginning of the flash, shall we approach the state where the amount of activated compound at the end of the flash is independent of the duration of the dark period. The results recorded in their second paper leave no doubt that the intensity of the flash in the experiments under consideration fell a good deal short of that required to give the maximum yield per flash. Experiments with intense flashes and yet higher concentration of carbon dioxide would yield information on the nature of the reaction or reactions involved in that stage of the whole process which goes on in the dark. According to Arnold (1933) it undergoes a simple breakdown at a rate proportional to its concentration. According to our schema the photochemical primary product may be activated chlorophyll which may lose its energy or hand it on to a compound of carbon dioxide. This activated compound, which might be called the photochemical product, can lose energy or combine with B and thus eventually give rise to the products of assimilation.

(2) *Temperature.*

We will discuss first the results of the experiments in which the yield per flash for different lengths of dark period was measured at high and low temperature. At 25° C. the amount per flash was practically the same with the dark period as short as 0·035 sec. as when the period was lengthened to 0·425 sec., but at 1·1° C. the assimilation per flash showed an increase over this range, the smallest value being 45 per cent. of the highest. The striking point is that with the long dark period the amount was the same at both temperatures. The interpretation they give is that the rate of breakdown of the photochemical product of the flash is increased by rise of temperature, but that the whole of the product gives rise to oxygen, their measure of assimilation. Loss of the photochemical product in any other reaction is assumed to be negligible. They admit that the results could be explained if the other reaction was not negligible, and the effect of temperature on its rate was the same as that on the breakdown of the product to oxygen. Although they produce no evidence in support of their view they reject this explanation " as an unlikely possibility". In terms of our schema the interpretation is as follows. After the light flash the S_l

formed may either lose energy at a rate of $k_4 S_l$ or may combine with B substance at a rate $k_5 S_l (B - X)$ and we have

$$-\frac{dS_l}{dt} = k_4 S_l + k_5 S_l (B - X),$$

$$\frac{dX}{dt} = k_5 S_l (B - X) - k_7 X.$$

The solution of these equations for the general case is complicated, but if we assume that dX/dt is relatively small, that is X is practically in equilibrium with S_l and equal to $k_5 S_l B/(k_7 + k_5 S_l)$, the solution is simplified. We can readily obtain the amount of S_l at the end of the flash, S_{l_0}, which is lost in the reaction $k_4 S_l$ and hence the portion A which is converted into products. When the dark period is very long

$$A = \frac{k_7 B}{k_4} \log_e \frac{k_7 (k_4 + k_5 B) + k_4 k_5 S_{l_0}}{k_7 (k_4 + k_5 B)}.$$

The authors state that with the intensity of flash used in these experiments the assimilation per flash is proportional to the intensity. If the intensity is so weak that the fraction of S_c activated is very small then S_{l_0} approaches to proportionality to the intensity. The assimilation is proportional to S_{l_0} and hence to the intensity when $k_4 k_5 S_{l_0}$ is very small compared with $k_7 (k_4 + k_5 B)$. Under these conditions

$$A = k_5 B S_{l_0}/(k_4 + k_5 B).$$

Our picture differs from that of the authors in that they assume that the whole of S_{l_0} gives rise to products, while according to us only a fraction does. It is obvious that since the yield per flash and the rate with continuous illumination do not continue to increase at the same rate as the intensity there must be a loss at some stage of the reaction of the energy absorbed; a loss which increases as the intensity increases. There is no change of the fraction of the incident energy which is absorbed. For such a loss their schema makes no allowance. According to our schema the loss is smaller the weaker the flash, the smaller S_{l_0} becomes, until $k_4/(k_4 + k_5 B)$ is lost and $k_5 B/(k_4 + k_5 B)$ is converted into products. With continuous illumination of very weak intensity only a very small fraction of B is in the form X, and the same fraction, $k_5 B/(k_4 + k_5 B)$, of the absorbed energy which is converted to S_l is utilised. This will be the efficiency of the process under these conditions if the energy absorbed by the chlorophyll is handed on to S_c (when only a small fraction of this is in the activated form S_l) more readily than it is lost.

Since the results of Warburg and Negelein (1923) suggest that the efficiency at low illumination is over 50 per cent. it would appear that $k_5 B$ is greater than k_4. Since change of temperature has practically no effect on the rate with low illumination it means that k_4 and k_5 are affected to the same extent. If this is the case then the fact that the yield per flash of low intensity for a long dark period is the same for low temperature as for high is at once explained.

The decrease with temperature of the yield per flash for shorter dark periods is explained by the decreased rate of combination of S_l with B to form X consequent

upon reduction in k_5 and a decreased rate of breakdown of X because of the smaller size of k_7.

(3) *Cyanide.*

Addition of HCN acts like reduction of temperature under these conditions of relatively high carbon dioxide and weak intensity of flash—reduced yield per flash except when the dark period is very long. We explained the effect on the assimilation with continuous illumination by assuming that HCN reduces the rate of breakdown of X. If combination of B with HCN was alternative to combination with S_l then the maximum yield per flash would be reduced, and the depressant effect in continuous illumination would be less instead of greater the higher the concentration of carbon dioxide and the intensity of illumination. The results fit in with the view that while HCN (or CN ion) is combined with X the latter is unable to give rise to products just as combination of the enzyme saccharase with Ag ions renders the complex of this enzyme and cane sugar incapable of giving rise to products of hydrolysis. The depressant acts like a reduction in the value of k_7, and as we have seen this does not affect the yield for weak flashes except when the dark period is not very long. It should reduce the yield for intense flashes, as an inspection of the expression on p. 470 will show. It is unfortunate that the authors made no investigation of this or indeed of any factor except change of chlorophyll content for flashes of high intensity.

(4) *Duration of dark period.*

The yield per flash is not in general a measure of the progress of the breakdown of X, since the shorter the dark period the more X and S_l is left at the end and hence the more X is available for the next dark period. Only in the limit, when the flash is so intense that the maximum amount of X is produced for long and short dark periods, is the yield a measure of the progress of the dark reactions. For our schema simplified for weak flashes the progress of assimilation in the dark would approximate to $X_0 e^{-k_7 d}$, with X_0 the amount of X formed as the result of the flash. This would be more nearly true the greater k_4 and $k_5 B$ are compared with k_7 (see p. 470).

If X_0 was independent of d (the duration of the dark period), as the authors assume when referring to the change of yield per flash with dark time as a measure of the progress of the Blackman reaction, then

$$\log_e \left(1 - \frac{A}{A_m} \right) = -k_7 d,$$

where A_m is the yield when d is very great and A when it is finite. With X_0 increasing as d is made smaller the yield for the short periods is greater than expected on the basis of the above expression, that is the calculated values of k_7 should fall as d increases. The data for $1 \cdot 1°$ C. show a fall from about 17 to 12.

(5) *Intensity of flash.*

This excess for the shorter dark periods should decrease as the intensity of the flash is increased until X_0 is equal to B for the short periods as well as the long. The authors did not carry out any experiments especially designed to compare the

change of yield with length of dark period at high and low intensities of flash. We have selected two of their many experiments, one at 6·9° C. with flashes of undiminished intensity and one at 7° C. when the light was passed through a " 50 per cent." filter. To render comparison easy we have plotted in Fig. 1 the yield per flash as a fraction of the yield with the longest dark period. As expected the relative yield for the shorter periods is greater when the intensity of the flash is reduced. The experiment with a 50 per cent. filter is the one done at the same time as the experiment with urethane (Fig. 1); another experiment showed a yet greater difference.
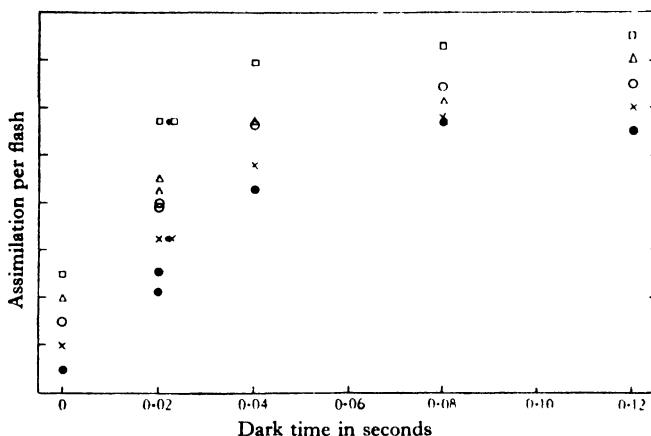

Fig. 1. Relation between assimilation per flash and length of dark period. In all cases the amount for a dark time of 0·12 sec. is plotted as unity. The position of the origin is indicated by the point for dark time zero. ● : low carbon dioxide (4·1 × 10⁻⁶ gram molecules per litre at 5·9 C. × : high carbon dioxide (71 × 10⁻⁶ gram molecules per litre) at 5·9° C. ○ : high carbon dioxide at 6·9° C. △ : high carbon dioxide at 7° C. plus phenyl urethane. □: high carbon dioxide at 7° C. plus 50 per cent. filter.

(6) *Concentration of carbon dioxide.*

On turning our attention to the relation between yield and concentration of carbon dioxide we may point out that in the above discussion we have neglected the question of regeneration of S_c during the dark period. Here we shall make the corresponding simplification of neglecting the amount of X and S_l left at the end of dark periods which are not very long. If the dark period is very long then the amount of S_c at the end will be $S \cdot C/(C + K)$, where C is the concentration of carbon dioxide and K is the ratio of the velocity constant of breakdown of S_c to that of its formation. As a result of the flash a definite fraction of this will be converted into S_l, which in its turn will give the appropriate amount of X. If C is very great then we shall have the same amount of S_c and hence the same amount of X for the short as for the long dark periods, but when C is not so great the value of S_c will be relatively smaller for the short periods. This factor therefore tends to work in the opposite way from that which we discussed in connection with the incomplete destruction of X in the short periods. That this latter predominates is indicated by the calculations referred to on p. 471. This carbon dioxide factor, as we may call it,

ought to reveal itself if we compare the yield as a function of dark period for high and low concentrations of carbon dioxide. Its presence is indicated in the curves in Fig. 1. A simple kinetic formulation of the authors' schema would lead to the same expectation.

Assuming that during the dark period

$$dS_c/dt = k_1 C \, (S - S_l - S_c) - k_2 S_c,$$
$$- dS_l/dt = k_4 S_l,$$

and during the light flash a fraction P of S_{cd}, the amount of S_c present at the end of the dark period, is converted into S_l by the flash, we can obtain an expression for the amount of assimilation per flash. This approaches $Pk_1CS/(k_1C + k_2)$, as d, the length of the dark period, approaches infinity. The fraction, P, increases with the intensity of the flash. The ratio, Q, of the assimilation per flash when d is finite to that when it is very great is

$$Q = \frac{1 - e^{-(k_1 C + k_2) d}}{1 - (1 - P) \, e^{-(k_1 C + k_2) d} - \dfrac{Pk_1 C}{k_1 C + k_2 - k_4} \cdot \dfrac{e^{-k_4 d} - e^{-(k_1 C + k_2) d}}{1 - e^{-k_4 d}}}.$$

As the intensity of the flash is increased this ratio is decreased, as the experimental results show (see p. 472). In other words, the length of the dark period for the assimilation to attain any given fraction of the maximum increases as the intensity of the flash is increased (cf. p. 475). When the concentration of carbon dioxide, and hence $k_1 C$, is increased the ratio rises, again in agreement with the results of the experiments.

The decrease with temperature of the yield when the dark period is not long is not due to the decrease of the velocity constant of the Blackman reaction alone, it depends also upon the decreased rate of recovery of the compound of S and carbon dioxide. As we saw on an earlier page the rate of assimilation in continuous illumination with 'ow concentration of carbon dioxide is determined mainly by the rate of formation of this compound; and there are suggestions that the rate under these conditions increases quite markedly with increase of temperature. For reasons which, on account of lack of space, cannot be presented now, we believe that with the higher concentration of carbon dioxide used by the authors the value of $k_1 C$ is so great as to make the recovery of S_c practically complete for the short dark periods even at the low temperature. But with lower concentration of carbon dioxide the recovery of S_c is apparently an important factor in determining the yield for the shorter dark periods.

We have given above (p. 472) the relation between S_c and C for long dark periods. The value of S_l at the end of the flash should be proportional to S_c at the beginning if the readjustment of S_c during the flash is negligible. If it were appreciable in a period of time as short as that of the flash, 10^{-5} sec., then the recovery of S_c would be complete even with the shortest dark periods which were more than a thousand times as long as the light flash. As we have seen when the concentration of carbon dioxide is low the evidence is against this. For the amount of X formed to be proportional to S_l, and hence to S_c, X must be a small fraction of B. That this is so is

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suggested by the authors' statement that with the intensity of flash used the yield was proportional to the intensity. No definite evidence is produced in support of this statement; but a comparison of the results in the first paper with those in the second where a variety of intensities was used gives some support. If this is the case then the relation between the yield per flash for long dark periods and C should be the same as that between S_c and C, that is the reciprocal of the yield should be a linear function of $1/C$. If X is more than a small fraction of B the slope of the curve should fall as the yield becomes greater, that is as $1/X$ approaches $1/B$. The values are plotted in Fig. 2 and suggest a value for K of about 6×10^{-6}. It should be noted that the values for C are those calculated by Warburg (1919) for a mixture of $NaHCO_3$ and Na_2CO_3, while the solution used in these experiments contained not sodium but potassium salts.
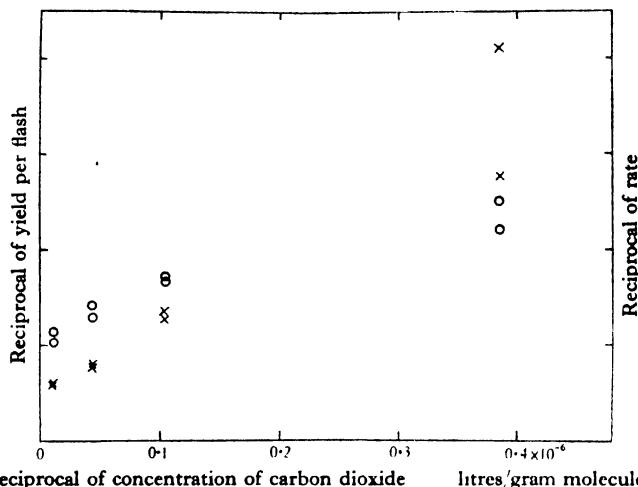

Fig. 2. Relation between assimilation and concentration of carbon dioxide. $\times$: reciprocal of rate. $\bigcirc$: reciprocal of amount per flash.

Exactly the same kind of relation between yield and C would be expected if we adopted the schema of the authors and gave the precise formulation as below. The only difference is that we should expect no departure at all from the linear relation in the values plotted. We do not feel that any definite conclusion on this point can be drawn from the data.

The conclusion the authors draw from their experiments is that the relation between yield and C is the same as that for rate and C in continuous illumination. If we formulate their schema as below it will be seen that we should not expect the same relation:

$$\frac{dS_0}{dt} = k_1 C (S - S_0 - S_1) - k_2 S_0 - k_3 I S_0,$$

$$\frac{dS_1}{dt} = k_3 I S_0 - k_4 S_1.$$

In the steady state

$$k_4 S_t = \frac{k_1 C k_4 S}{k_1 C (1 + k_4/k_3 I) + k_4 (1 + k_2/k_3 I)}.$$

For continuous illumination the value of C when the rate $k_4 S_t$ is half of the maximal rate is $\dfrac{k_4 (k_2 + k_3 I)}{k_1 (k_4 + k_3 I)}$, whereas when the yield is half the maximal value obtained with a high concentration of carbon dioxide then $C = k_2/k_1$ as with our schema. The value with continuous illumination varies between k_2/k_1 when the intensity, I, is very low, and k_4/k_1 when it is very high. We do not know the intensity of illumination used in the experiments, but it is clear from the results (see Fig. 2) that the value of C for half maximal rate is higher than that for half maximal yield. Our more complicated theory gives the same relation between rate and C when the intensity of illumination is made very weak as does the authors' simpler schema. Consequently it is of interest to compare the values of k_2/k_1 from experiments in weak continuous illumination with the above estimate from experiments in flashing light (about 6×10^{-6}). The experiments of Van den Honert (1930) are complicated by diffusion, but he concludes that the value is certainly less than 10^{-5}. Harder's (1921) experiments with the lowest illumination do not give enough data, but those with the next lowest (667 metre-candles) indicate a value about 10^{-5}. The temperature in these experiments was higher than in those with flashing light.

(7) *Phenyl urethane.*

In the experiments in which the effect of phenyl urethane was examined comparison was not made with material in the same conditions except for the addition of the urethane but with the light transmitted through a "50 per cent." filter. As stated earlier this reduction of the intensity reduces the yield per flash more for long dark periods than for short. It so happened that the addition of the urethane had practically the same effect as had the reduction of intensity on the yield for the long dark period. If, as the discussion of the effect of urethane on assimilation in continuous illumination suggested (cf. p. 467), this substance acts like decreased intensity of illumination, then we should expect the same reduction of yield per flash for short dark periods as for long. Actually the results (see Fig. 1) indicate a greater reduction as the dark period is shortened. This suggests that the urethane acts in yet other ways. As we have seen reduction of temperature, of concentration of carbon dioxide, or addition of HCN causes a greater reduction of the yield for short dark periods.

(8) *Maximum yield and intensity of flash.*

In considering the results of the experiments in which the intensity of the flash was varied the first point to be noted is that we have no direct evidence that the dark period is in all cases long enough to give the maximum yield. In the earlier experiments where the yield was stated to be proportional to the intensity of the flash 0·035 sec. was long enough at 25° C., and here the authors tacitly assume that 1/21 sec. is long enough for all intensities of flash. From a comparison of the results

of the earlier experiments with those under consideration we estimate that the intensity in the former was between one-tenth and one-third of the highest intensity of the latter. According to the simpler schema of the authors, as we have already formulated it, we should expect a longer dark period to be necessary for the recovery of S_c when the intensity of the flash is great (see p. 473). Quite apart from any theory experimental evidence is obviously required on this point.

As the intensity of the flash was increased the yield increased at first rapidly and then more and more slowly until a maximum value was attained. According to the authors' formulation the rate of activation of S_c is proportional to the intensity of illumination and to the amount of S_c unactivated, and hence the relation between the yield per flash, P, and the intensity of the flash, I, should be as follows,

$$\log_e \left(1 - \frac{P}{P_m} \right) = -kI,$$

where P_m is the yield when the intensity is made very great. As the authors have to admit, use of a value for P_m anywhere near the highest value recorded gives a curve of $\log (1 - P/P_m)$ against intensity of flash which has a slope which increases with intensity instead of remaining constant.

According to our schema as formulated on p. 470 the yield for long dark periods should be related to S_{l_0} as follows:

$$\frac{k_7 B}{k_4} \cdot \log_e \frac{k_7 (k_4 + k_5 B) + k_4 k_5 S_{l_0}}{k_7 (k_4 + k_5 B)}.$$

Even for flashes so weak that S_{l_0} increases proportionally with intensity, that is when only a very small fraction of S_c is activated, the yield falls away from proportionality to intensity as soon as S_{l_0} ceases to be small compared with

$$k_7 (k_4 + k_5 B)/k_4 k_5,$$

and when S_{l_0} is relatively great then the yield increases as the logarithm of S_{l_0}, which at this point is increasing less rapidly than the intensity of the flash. To give a numerical illustration, if k_4 is equal to $k_5 B$ (see p. 470) and ten times as great as k_7, then when S_{l_0} is as great as $2000B$ the yield would be only $0.92B$. If any such value is taken as the maximum yield then the curve of $\log (1 - P/P_m)$ shows an increasing slope. The same result would be expected on the basis of the authors' simpler theory if the dark time was not long enough for the completion of the dark reactions.

The suggestion of the authors that the relation between the rate of photosynthesis and the intensity of continuous illumination is of the same nature as that between yield and intensity of flash can only be excused by the fact that they give very little consideration to a kinetic formulation of their schema.

(9) Amount of chlorophyll.

Chlorella cells with different chlorophyll content were obtained by culture in different coloured light using mercury vapour and neon lamps. The cells may well differ in other components of the assimilatory mechanism which are measured with greater difficulty than is the chlorophyll. The assimilation was measured in bright

continuous illumination and in flashing light of high intensity. It is stated that it was not possible in all cases for cells with low chlorophyll to obtain a continuous illumination of high enough intensity to give the maximal rate of assimilation. There is the same difficulty with leaves of yellow varieties as compared with those of green (Willstätter and Stoll (1918)). The explanation suggested by the authors is that the cells with low chlorophyll have a greater capacity for the Blackman reaction. On no occasion do they give any indication as to what precisely is meant by capacity for reaction. Perhaps they mean the concentration of some catalyst, to the concentration of which the speed of breakdown of activated S_c is proportional. This is Baly's (1934) suggestion (cf. p. 462). According to our schema the phenomenon can be accounted for without postulating that the cells poor in chlorophyll are richer in any factor. If chlorophyll is acting as a sensitiser then a greater intensity of illumination
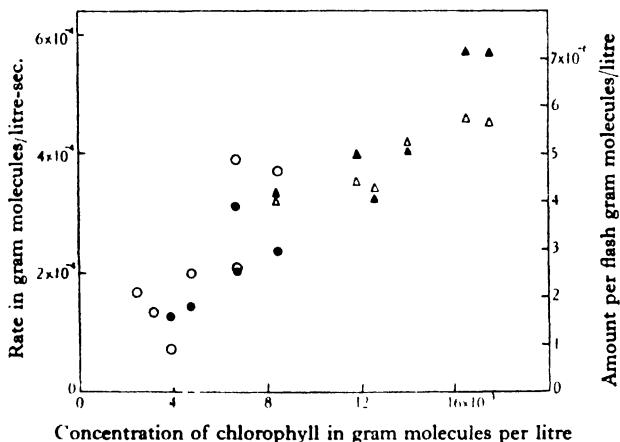

Fig. 3. Assimilation for cells of different chlorophyll content. Circle for cells grown under neon lamp. Triangle for cells grown under mercury vapour lamp. Solid for amount per flash, others for rate in continuous light.

will be required when the sensitiser is small to bring S_t up to the value to maintain the whole or any given fraction of B in the X form.

No mention is made of any difficulty being experienced in obtaining the maximum yield in flashing light with the cells poor in chlorophyll. Information on this point is important.

Turning to the results which are presented in Figs. 3 and 4 we may note that the authors' claim that the yield per flash shows a proportionality to chlorophyll while the rate falls away from proportionality. In other words, the ratio of rate to yield falls with increase of chlorophyll. The correlation coefficient for ratio and chlorophyll is only 0·14, much too small to be significant. This, of course, only means that the values are much too scattered to be expressed by a straight line relation. When the results for the cells grown under the neon lamp (low chlorophyll) are considered separately from those for cells grown under the mercury lamp, it is seen that the cells with least chlorophyll have the lowest value for the ratio and those with most the

highest ratio, while the cells grown in mercury light suggest a slight fall of the ratio
with increase of chlorophyll (see Fig. 4).

According to our schema the assimilation in high continuous illumination with a
high concentration of carbon dioxide should be proportional to B if there is enough
S to saturate B under these conditions. The yield per flash, however, depends upon
both B and S, S_{t_0} being proportional to S (see p. 470). If B was kept constant and S
increased with chlorophyll then the ratio of rate to yield would fall (as with cells
grown under the mercury lamp), if B increased as rapidly as S the ratio would fall,
but not so rapidly, while if B increased so that $(k_4 + k_5 B)$ increased more rapidly than
S then the ratio would rise. A scatter of the values for the ratio would result from a
variability of the ratio of B to S.

It is clear that our schema is sufficient to explain the general form of the results if
we assume that S increases when chlorophyll does, and we have no need to postulate
any accompanying decrease of any other part of the mechanism. The ratio of the
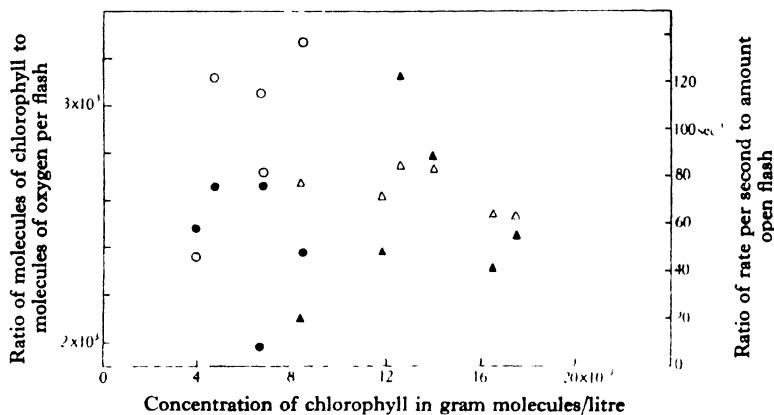

Fig. 4. Ratios of (a) molecules of chlorophyll to molecules of oxygen per flash (indicated by solid)
and (b) rate per second to amount per flash for cells with different chlorophyll. Triangle for cells
grown under mercury vapour lamp. Circle for cells grown under neon lamp.

number of chlorophyll molecules present to the number of molecules of oxygen
produced per flash varies between 2000 and 3000 (see Fig. 4). According to the
authors this may indicate the ratio of the number of chlorophyll molecules present
to those effective in combining with carbon dioxide. According to our schema,
since we have no evidence as to whether the whole of B is converted into X after a
flash or that the whole of X is decomposed, it is the upper limit to the ratio of
chlorophyll molecules to B units.

If the maximal yield per flash approaches B and the maximal rate $k_7 B$ with high
chlorophyll content then the value of k_7, the velocity of the Blackman reaction
would appear to be about 60 or 70 at 25° C. In experiments carried out at lower
intensities the ratio of rate to yield is between 85 and 90 at the same temperature.
This, however, has little significance, since we have no information as to the relative
values of the illumination for flashing and continuous light.

The authors find it difficult to reconcile the large ratio of number of molecules of chlorophyll to molecules of oxygen reduced per flash with the high efficiency of assimilation per absorbed energy reported by Warburg and Negelein (1923) when using a weak intensity of illumination. According to our schema the explanation is as follows. With high carbon dioxide S tends to be wholly in the form S_t, the energy absorbed by chlorophyll, the sensitiser, is much more readily handed on to S_c, thus activating this, than lost to other molecules; practically the whole of B being available the activated S_c, or S_t as we have called it, passes into the form X much more readily than it loses energy.

If the number of molecules of oxygen produced per flash is a measure of the number of effective centres, as it would be on the theory suggested by the authors, then we can make an estimate of the order of the velocity constant of the combination of carbon dioxide with these centres. In one of the experiments with cells with high chlorophyll the concentration of centres is $7 \cdot 1 \times 10^{-6}$ gram units/litre, and the rate of assimilation with a concentration of carbon dioxide of 91×10^{-6} gram molecules/litre is $4 \cdot 5 \times 10^{-4}$ gram molecules/litre-second. On the basis of Warburg's experiments (1919) we estimate a rate of $0 \cdot 54 \times 10^{-4}$ with a concentration of $0 \cdot 53 \times 10^{-6}$, at which concentration the rate is practically proportional to the concentration, and hence a small fraction of the centres is occupied by carbon dioxide. If the whole of the carbon dioxide, whether hydrated or not, was effective this would indicate a velocity constant for the combination of carbon dioxide with the centres of the order of $1 \cdot 4 \times 10^{7}$ litres gram molecule-second. Since the H_2CO_3 is only about $1/500$ of the total carbon dioxide, the constant would be increased to 7×10^{9} if only the hydrated molecules were effective. If the assimilatory centres are practically stationary the calculated velocity constant, assuming that collisions of the carbon dioxide molecules are effective if they strike a point centre within a circle of radius equal to that of a carbon dioxide molecule, is of the order of 4×10^{9}. This is based on the assumption that the molecules behave as if they were in a gas. There are suggestions that the constant may be as much as ten times as big in a liquid (Moelwyn-Hughes, 1933). Although these figures indicate only the order of the magnitude they suggest that if H_2CO_3 is the molecule concerned then practically every collision is effective, and if CO_2 about one in a thousand. If the temperature coefficient of the assimilation at low carbon dioxide is as high as 4 (see p. 466) this suggests that a very small fraction of the collisions is effective—something of the order of 10^{18}. We cannot discuss the situation in detail now and can only indicate the possibilities. The centres may be much more numerous than we have assumed in our calculation, but a concentration of 10^{18} times as many would mean 10^{13} gram units/litre. The effective area of the centres may be much greater, but this would mean an area per unit greater than that of the cell and there are 10^{5} units per cell. Although according to our schema there probably are many more S units than assumed by the authors we think that part of the difficulty lies in the probability that the estimated temperature coefficient is too high. It would have to be as small as $1 \cdot 3$ if about one collision in a thousand were effective.

(10) *Pretreatment with ultra-violet radiation.*

Recently Arnold (1933) has reported that the maximum rate in continuous illumination and the maximum yield in flashing light are reduced to the same extent by previous exposure to ultra-violet light. He produces evidence that the chlorophyll is not changed chemically by this treatment. According to our schema a destruction of S should, if S is originally big enough, first decrease the yield more than the rate and then as more S is destroyed the rate more than the yield. A destruction of B would have the same effect on rate and yield if S were big enough to saturate B for continuous and flashing light. Experiments with different exposures to ultra-violet light would be helpful in ascertaining the nature of its effect.

IV. COMPARISON WITH EARLIER EXPERIMENTS.

In the experiments which we have been discussing the yield per flash of high intensity at 25° C. is between 1 and 2 per cent. of the assimilation per second in continuous illumination of high intensity. The duration of the dark period in the experiments with flashing light was about 1/10 sec. Warburg (1919) found with intermittent illumination consisting of equal periods of light and dark that the assimilation per second of light was greater than with continuous illumination; as the intermission was made more rapid the former approached to being twice the latter. Expressed in terms of the excess for one period of light over the assimilation in the same time of continuous illumination the excess approached zero as the period was shortened, and as the period was lengthened it increased, in one experiment, from 1·1 per cent. for a period of 0·015 sec., to 8·4 per cent. for 0·15 sec., to 54 per cent. for 1·5 sec. and then to 210 per cent. for 15 sec. In another experiment the values were 0·37 per cent. for 0·0038 sec., 2·9 per cent. for 0·038 sec. and 17·4 per cent. for 0·38 sec. The unit is the amount of assimilation in 1 sec. of continuous illumination. These experiments were at the same temperature as those we discussed earlier. Even with the dark periods of the same order of length as those in the experiments of Emerson and Arnold (1933) the excess is greater than the amount of assimilation per dark period recorded by these authors, and for longer dark periods it approaches being a hundred times as much. The latter values are very much affected by the accuracy of the values for assimilation in continuous illumination, since with long periods there is only a small difference between the rate for intermittent and continuous illumination.

The essential difference between the procedure in the two cases is that in one the duration of the light period was very short and constant and in the other much longer, even at its shortest, and varied in length with the dark period. Emerson and Arnold (1933) state that the yield per flash was practically the same if the flash was completed in 10^{-5} sec. or spread over a period a hundred times as long. There are no details of the experiment, and even then the period is shorter than the shortest light period in Warburg's experiments. A complete solution of the problem must await further experimentation. In the first place we want to know if the dark period in the experiments with flashing light was long enough for the completion of the

dark reactions, and then, if the yield could be increased by lengthening the period, could it be further increased by increasing the intensity of the flash. Experiments with light periods intermediate in length between those of Warburg and those of Emerson and Arnold are essential.

If the results of Emerson and Arnold do really indicate the maximum assimilation in the dark period that can be attained with the conditions of light and carbon dioxide used then we are left in the position that much of the excess recorded by Warburg must be due to the rate of assimilation during the light period of intermittent illumination being greater than in continuous illumination. Warburg (1919) suggests that the whole of the excess is to be attributed to this cause. His explanation is that a compound of carbon dioxide is reformed during the dark and has a higher average concentration during intermittent illumination than during continuous. He advances no real reasons for this view. On his formulation or any that we have considered this explanation could only hold if the concentration of carbon dioxide was such that an increase in the concentration resulted in an increase in the rate with continuous illumination. Moreover, since, with rapid intermission, the rate during the light period would have to be practically twice that with continuous light it ought to be possible to secure twice the rate under the latter conditions by increasing the concentration of carbon dioxide. Actually Warburg (1919) claims that the concentration is so high that the rate is almost maximal. Emerson and Arnold (1933) used a similar concentration and temperature, but we have no precise information of the illumination in either case. If the concentration of carbon dioxide was so high as to give almost the maximal rate then only a part of the excess recorded by Warburg can be attributed to higher rate during the light period.

Although the experiments of Emerson and Arnold have provided us with much useful information, this comparison with the data of other workers shows it to be lacking at critical points. If this analysis has raised these questions in a concrete form, besides providing a picture of the mechanism of photosynthesis which gives a new significance to these and other data, then it will serve its purpose.

V. SUMMARY.

We have shown that the effect of such factors as concentration of chlorophyll, concentration of carbon dioxide, intensity of illumination, and poisons, on the rate of photosynthesis in continuous illumination can be interpreted on the basis of a mechanism of photosynthesis suggested in an earlier paper. Other suggested mechanisms are shown to be inadequate.

The results of the recent experiments of Emerson and Arnold with flashing light have been shown to be in harmony with the schema, but further experiments are necessary before the full significance of the results can be ascertained. This necessity has been emphasised by a comparison of the results with the earlier work of Warburg with intermittent illumination.

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LA CHRONAXIE EN BIOLOGIE GÉNÉRALE

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TABLE DES MATIÈRES.

I. LE CONCEPT DE CHRONAXIE; SA GENÈSE.

LES sujets habituels de la physiologie expérimentale, Lapin, Chat, Chien, ou d'autre part, Grenouille, sont des animaux à mouvements rapides et à excitabilité rapide, du moins pour leurs nerfs et leurs muscles de la vie de relation. L'Homme est dans le même cas. Les durées qui interviennent dans l'excitation électrique sont si brèves sur ces nerfs et ces muscles que la physique du siècle passé ne réussissait pas à les atteindre. Pour les étudier, Fick (1863) eut l'idée de chercher un muscle dont la contraction serait plus lente, afin d'avoir affaire à des temps d'excitation plus longs. Il avait donc à priori l'idée d'une relation chronologique entre la contractilité et l'excitabilité. Et en effet, sur le muscle adducteur des valves de la grande moule d'eau douce (*Anodonta cygnea*), il vit facilement que le courant devait, pour exciter, être plus intense avec des passages courts qu'avec des passages longs. Sur le nerf de la grenouille, Du Bois-Reymond, utilisant un appareil mécanique pour réduire jusqu'à un demi-centième de seconde la durée du courant, n'avait pu observer aucune diminution d'efficacité; donc, disait-il, le passage du courant constant est sans aucune importance, ce qui vérifiait sa loi fameuse: "l'excitation est fonction de la dérivée de l'intensité par rapport au temps." Cette formule implique en effet que l'action du courant est terminée du moment qu'il est arrivé à sa pleine intensité.

Fick avait pour but de démontrer que cette loi était fausse; il n'insista pas sur la liaison de l'excitabilité lente à la contractilité lente, cette liaison lui paraissant sans doute aller de soi. Engelmann quelques années plus tard (1870) partit du même principe quand il voulut préciser la façon dont l'intensité du courant doit s'accroître pour redevenir efficace lorsqu'on diminue la durée du passage; il s'adressa

à un muscle lent de Mammifère, un muscle lisse, l'uretère du Lapin, et put établir une loi quantitative en comptant les durées de passage par quarts de seconde. Mais il insista aussi sur ce fait que, seuls, les organes à contraction rapide répondent à un courant électrique bref; il y a pour chacun, dit-il, un "temps physiologique".

L'expression est significative; bien qu'Engelmann n'ait pas pris la peine de la développer, l'idée est manifestement celle de la chronaxie, telle que je l'ai définie 40 ans plus tard, "une valeur du temps" propre à chaque espèce de cellule et réglant tous les processus vitaux de celle-ci.

Mais cette conception était liée à la démonstration de l'efficacité du courant électrique en régime constant; s'il était admis que les quarts de seconde qui comptent manifestement dans l'excitation de l'uretère représentent pour ce muscle lent les durées de passage mille fois plus petites que seule l'imperfection de la technique avait empêché de saisir dans l'excitation du muscle strié et de son nerf, alors la loi de Du Bois-Reymond était fausse. Hérésie inadmissible!

Du Bois-Reymond régentait l'électro-physiologie mondiale; bien que Fick comme Engelmann fussent des physiologistes notoires, souvent cités avec respect pour d'autres travaux, ceux-ci tombèrent dans l'oubli. Trente ans plus tard, Georges Weiss (1901) les ignorait quand il reconnut sur le sciatique de la Grenouille que l'intensité liminaire du courant électrique varie systématiquement avec la durée du passage entre 3 millièmes et 3 dix-millièmes de seconde. Je les ignorais également au point de considérer le résultat de Weiss comme paradoxal et de chercher diverses interprétations pour sauver la loi de Du Bois-Reymond.

Mais en reprenant les expériences de mon côté (1903 *a*) j'observai, de la Grenouille au Crapaud, des durées efficaces un peu plus longues; Grützner et ses élèves (Schott, 1891) avaient soupçonné une différence, mais enlisés dans la théorie de Du Bois-Reymond, ils avaient fait fausse route. D'ailleurs, entre le gastrocnémien du Crapaud et celui de la Grenouille, l'allongement de la durée de la secousse est relativement peu de chose. Pour avoir une gradation plus marquée dans les temps d'excitation, j'allai chercher parmi les animaux marins, des muscles à contractions notablement plus lentes. On voit que je répétais, sans le connaître, le raisonnement de Fick, ou plutôt son intuition, car pas plus que lui, je ne discutais une relation qui me paraissait s'imposer.

L'intuition était juste; elle se vérifia dès ces premières expériences (1903 *b*), comme elle s'est vérifiée dans tous les cas qui ont été examinés par la suite. La Tortue, qui avait paru faire exception, est rentrée dans la règle quand on a rectifié une erreur affectant le chiffre donné d'abord (L. et M. Lapicque, 1927 *b*).

En mettant à part certains muscles d'Invertébrés à fonction particulière, les *muscles à cliquet* (Sperrmuskel d'Uexkull) qui présentent un raccourcissement permanent, les muscles, en général, répondent à un stimulus unique par une contraction élémentaire dont on peut, en première approximation, donner une seule et même description générale; un raccourcissement progressif atteignant un maximum auquel succède aussitôt un relâchement. Pour le gastrocnémien de la Grenouille, le phénomène tout entier se passe en un clin d'œil; enregistré sur un cylindre tournant assez vite, il montre une durée totale de 10 à 20 centièmes de

seconde. Le pied de l'Escargot met plusieurs secondes à accomplir un mouvement analogue qui nous paraît très lent; il donnera pourtant un graphique semblable, à condition de l'inscrire sur un cylindre tournant cinquante ou cent fois plus lentement. De même l'estomac de la Grenouille mettra un temps de l'ordre de la minute pour effectuer le phénomène contractile homologue. Nous avons donc, pour les divers muscles, des contractilités qui diffèrent essentiellement par la longueur du temps qu'elles occupent.
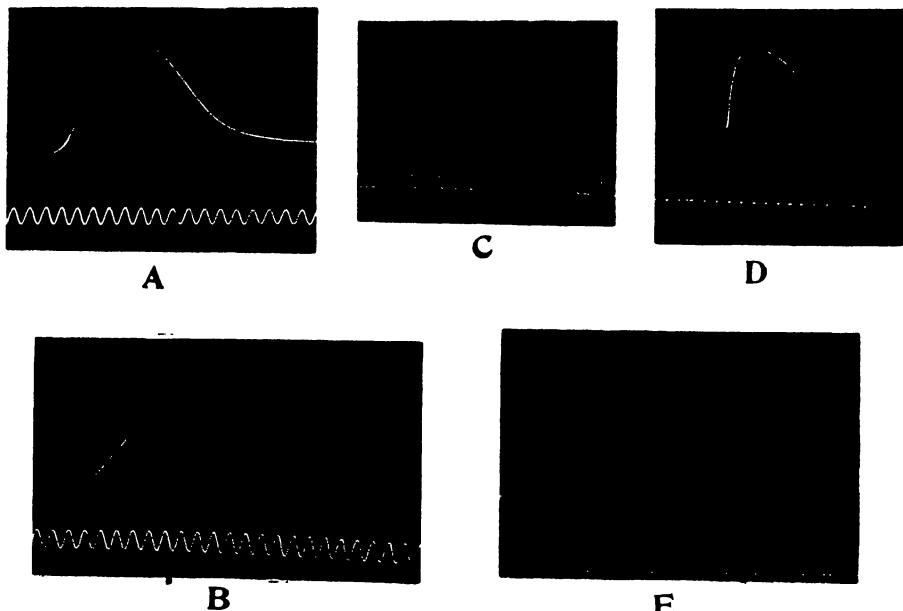

Fig. 1. Graphiques de contractions élémentaires de divers muscles. A, gastrocnémien de Grenouille, temps en cinquantièmes de seconde. B, gastrocnémien de Crapaud, mêmes unités de temps. C, ventricule du cœur de Grenouille, temps en cinquièmes de seconde. D, rétracteur du pied de l'Escargot, temps en secondes. E, estomac de Crapaud (exceptionnellement rapide), temps en secondes.

Mais cette ressemblance n'apparaît que si la stimulation de son côté est affectée du même facteur chronologique. La bobine d'induction était naguère employée comme appareil de stimulation passe-partout (nombre de physiologistes contemporains commettent encore cette erreur); l'onde induite d'ouverture (break shock) a une durée de l'ordre du millième de seconde. C'est parfait pour les nerfs et les muscles rapides de la physiologie classique; c'est trop bref pour les muscles lents; il en résulte chez ceux-ci après un raccourcissement incomplet, une contracture qui prolonge énormément la descente de la courbe. Pour mettre en jeu normalement ces muscles lents, il faut employer un stimulus électrique durable, décharge d'une capacité assez grande, ou, suivant les cas, passage de courants de l'ordre du dixième de seconde et davantage.

De même, si l'on recherche, pour divers temps de passage de courant, quelle intensité sera nécessaire pour obtenir la plus petite contraction perceptible (seuil de l'excitation), la courbe représentative est toujours la même, à condition de prendre dans chaque cas une échelle convenable pour les temps, d'une part, pour les intensités, de l'autre.

Voici quelques exemples sous forme graphique (Fig. 2). La ressemblance a été obtenue en faisant partout égale à 1 l'intensité liminaire invariable pour les temps longs (rhéobase); d'autre part, en rétrécissant l'échelle des durées à mesure que le muscle était plus lent; la longueur d'abscisse cotée 1 σ (millième de seconde) pour le gastrocnémien de Grenouille, a été cotée 8 σ pour le cœur du même animal, et
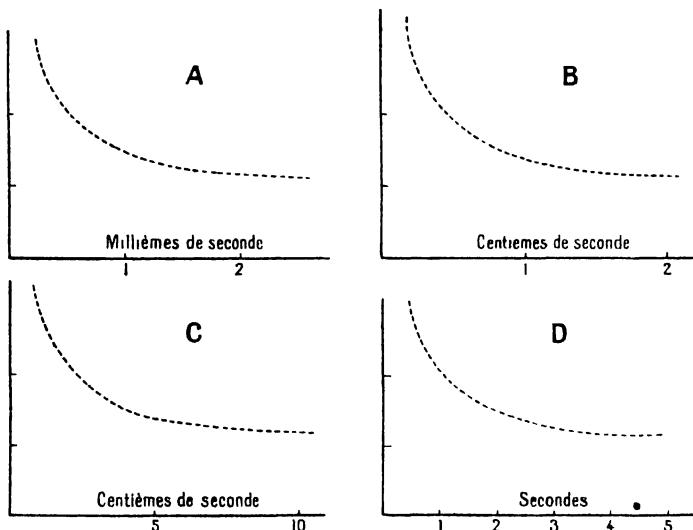

Fig. 2. Courbes de l'intensité liminaire en fonction de la durée du stimulus. A, pour le gastrocnémien de la Grenouille. B, pour le ventricule cardiaque de la Grenouille. C, pour le rétracteur du pied de l'Escargot. D, pour l'estomac de la Grenouille. Chacune sur une échelle des durées appropriée.

deux secondes entières pour son estomac; enfin 40 σ pour le rétracteur du pied de l'Escargot. Ces diverses valeurs d'abscisse exigées par les divers muscles pour figurer convenablement leurs excitabilités les rangent dans le même ordre que leurs durées de contraction.

Par ces quelques exemples concrets, on voit bien qu'il y a pour chaque muscle une valeur particulière du temps réglant la fonction en même temps que l'excitabilité. Cette notion est, au sens large, la *chronaxie*.

La durée de contraction n'est pas susceptible d'une mesure précise; elle n'est même pas constante pour un muscle donné. Au contraire, les temps impliqués dans l'excitation sont bien définis; un ajustement rigoureux des échelles des temps est possible, et alors on voit se superposer les résultats numériques d'expériences sur des muscles divers; les points expérimentaux se placent sur une seule et même courbe.

La Fig. 3 représente les chiffres donnés par 5 expériences réelles, chacune d'après son échelle de temps particulière indiquée dans le bas, les intensités liminaires observées étant d'autre part dans chaque cas divisées par la rhéobase correspondante. On voit qu'on peut interpoler d'une façon satisfaisante toutes les expériences à la fois par une seule courbe telle que celle qui est représentée en pointillé. Si nous avions une théorie complète et exacte du processus de l'excitation électrique, nous en obtiendrions l'expression mathématique d'une telle courbe ; cette expression comprendrait nécessairement une *constante de temps*, qui serait la chronaxie rationnelle. J'ai perdu beaucoup de travail à la chercher sans succès ; je n'ai même que tardivement (1925) trouvé une formule empirique (et encore bien
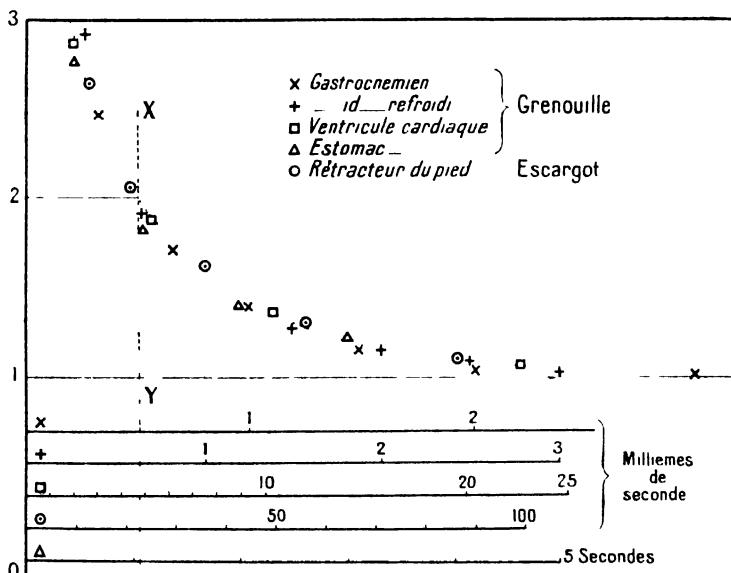

Fig. 3. Cinq expériences réelles superposées par ajustement des échelles de durées

compliquée !) pour traduire la courbe avec une approximation suffisante. L'hyperbole proposée par Weiss, ou l'exponentielle proposée par divers auteurs, dont moi-même à un certain moment, donnent par rapport à l'expérience des écarts systématiques assez grands pour constituer parfois des erreurs gênantes. C'est pourquoi je me suis résigné à un paramètre chronologique empirique.

Sur la Fig. 3, on voit bien qu'une grandeur quelconque du temps prise sur l'une des échelles correspond sur chacune des autres échelles à une valeur définie qu'on pourra lire en élevant par le point d'abscisse choisi une ordonnée qui coupe toutes les échelles ; cette ordonnée coupera aussi la courbe commune en un point qui peut être défini sur cette courbe par sa hauteur au-dessus de la rhéobase. Si nous avions les diverses expériences figurées séparément, comme dans la Fig. 2, chacune sur l'échelle qui convient à sa figuration, nous pourrions retrouver le point en question par cette considération de hauteur. Convenons que nous prendrons toujours la

hauteur 2, c'est-à-dire l'intensité liminaire double de la rhéobase; ce sera l'opération équivalente au tracé de ligne XY sur la Fig. 3, et sur chaque échelle, soit séparée, soit juxtaposée aux autres, nous pourrons lire une valeur d'abscisse représentant une durée caractéristique mesurable avec une grande précision; ici, en chiffres ronds, un demi-millième de seconde pour le gastrocnémien, 4 millièmes pour le cœur, 22 millièmes pour l'Escargot, 1 seconde entière pour l'estomac. Ces valeurs sont des chronaxies *senso strictu*, d'après la définition que j'en ai donnée en 1909.

Remarquons ceci: quand on est dans les conditions normales capables de donner pour la relation intensité-durée des valeurs qui suivront la courbe générale, il est inutile de tracer cette courbe; il suffit de: 1º déterminer par tâtonnement la rhéobase; 2º régler son instrumentation pour obtenir sur l'objet interrogé une intensité double; 3º déterminer par tâtonnement la durée de passage de courant pour laquelle on sera de nouveau au seuil de l'excitation. Cette durée est la chronaxie, qu'on obtient ainsi directement, sans aucun calcul, à l'état de grandeur expérimentale.

Telle est la forme simplifiée sous laquelle la chronaxie s'est vulgarisée dans les laboratoires de physiologie et les cliniques neurologiques, et parfois on oublie qu'elle n'est qu'une mesure pratique de chronaxie au sens large, un moyen de saisir dans le phénomène artificiel de la stimulation électrique, la façon lente ou rapide dont le temps conditionne les processus élémentaires pour chaque espèce de matière vivante.

II. GÉNÉRALITÉ DU CONCEPT; RELATION À LA FONCTION.

Cette signification est générale. Le cas du muscle a le premier révélé une relation entre la vitesse d'excitabilité et la vitesse fonctionnelle, car sous forme de contraction, celle-ci tombe directement sous le sens. Mais il y a aussi des chronaxies nerveuses très diverses suivant le nerf considéré, et dans la comparaison de nerf à nerf, la durée d'une onde d'influx varie comme la chronaxie, la vitesse de transmission en sens inverse.

La relation se retrouve chez les protozoaires. Il y a longtemps qu'avec Fauré-Frémiet (1913), sur une Vorticelle, infusoire pédiculé bien connu qui se rétracte avec une grande vivacité quand on l'irrite d'une manière quelconque, nous avons étudié la stimulation électrique; 1º la courbe intensité-durée liminaires présente la forme typique sur laquelle j'ai insisté plus haut, donc il est légitime d'en déduire une chronaxie; 2º cette chronaxie est petite, 2 à 3 σ, ce qui est en accord avec le caractère rapide de la réponse.

La relation se retrouve encore chez quelques végétaux. Sur les Spirogyres, algues submicroscopiques dont chaque cellule contient un filament vert spiralé, à la suite d'irritations diverses, ce filament se resserre sur lui-même d'une façon si lente qu'il faut repérer des positions successives pour s'en apercevoir; le début de cette contraction peut être provoqué d'une façon réversible par un courant électrique plus ou moins intense passant un temps juste suffisant; la courbe intensité-durée ainsi déterminée est encore de la même forme, mais avec des temps comptés en dizaines de seconde.

J'ai publié (1926, p. 224) une figure montrant une série de points ainsi obtenus se superposant exactement à la courbe de l'Escargot, mais il a fallu prendre une échelle des temps telle que 8 secondes correspondissent à 22 millièmes de seconde, pour l'Escargot.

Plus directe est la comparaison que j'ai pu faire au Brésil entre deux espèces de Sensitives. L'espèce ordinaire, *Mimosa pudica*, rabat sa feuille et resserre ses folioles d'un mouvement très vif; cette réponse bien connue aux stimulations mécaniques peut aussi être obtenue au moyen d'un courant électrique, avec une cathode fixée à demeure sur le coussinet moteur ; la courbe intensité-durée liminaire présente encore cette fois la forme décrite, et fait ressortir légitimement des chronaxies de l'ordre du centième de seconde. Une autre espèce, *Mimosa velosiana*, porte des folioles bien plus grandes dont le mouvement s'effectue relativement lentement. La stimulation électrique fait ressortir des chronaxies se comptant en dixièmes de seconde. Les deux chronaxies sont entre elles sensiblement comme les durées des mouvements, ainsi que les chronaxies et les durées de contraction pour deux muscles ayant entre eux une différence de vitesse du même ordre.

Mais les organes moteurs des Sensitives ne sont pas des muscles ; la relation qui s'observe entre deux organes de même sorte n'est pas valable d'une sorte à l'autre. Le *Mimosa pudica*, avec son mouvement rapide, a une chronaxie d'Escargot, animal lent.

C'est que la contractilité musculaire appartient à une différenciation interne du muscle, la contraction y est une conséquence *secondaire* et spécialisée de l'excitation, tandis que le mouvement de la Sensitive est une conséquence directe du processus général de l'excitation.

Une part importante de ce processus est devenue intelligible au point de vue physicochimique, en interprétant une série de données classiques au moyen des recherches de Nernst et de Ralph Lillie. La surface d'une cellule saine et en repos est toujours chargée, à un taux défini, d'électricité positive. Sous l'influence de n'importe quelle irritation effective, cette polarisation diminue, produisant une variation passagère de potentiel. Accessible depuis longtemps à nos instruments de physique, cette perturbation électrique est bien connue, pour le nerf, pour le muscle, comme pour le coussinet moteur de la Sensitive, sous le nom de *courant d'action*. Elle apparaît de la même façon sur les cellules qui ne manifestent aucune réaction mécanique ; Waller a insisté sur sa généralité, en montrant que ce "blaze current", comme il l'appelle, est un signe fondamental de la vie. Disons plutôt que c'est un signe de la mise en jeu de l'irritabilité protoplasmique. Sur des cellules banales, telles que le parenchyme d'une feuille d'arbre, on peut, en le prenant comme test, rechercher l'intensité efficace du courant électrique en fonction de sa durée ; on retrouve aussi exactement que le comporte la précision de l'expérience, la loi que nous connaissons pour la mise en jeu d'un mouvement où la motilité existe (L. et M. Lapicque, 1927 *a* ; D. Auger, 1927). Sur le nerf, l'influx nerveux n'est autre que cette perturbation électrique transmise de proche en proche au long de la fibre nerveuse. Sur le muscle, il y a aussi transmission d'une sorte d'influx le long de la fibre musculaire, et c'est après lui que se propage la contraction.

D'autre part, toute une série d'auteurs, dans des expériences variées sur les objets les plus divers, ont montré que l'état d'excitation s'accompagne toujours d'une augmentation de la perméabilité cellulaire. La surface cellulaire, en effet, est à l'état normal, peu perméable; c'est pourquoi on la considère classiquement comme constituée par une membrane semi-perméable. L'existence matérielle d'une telle membrane ne me paraît pas soutenable; je pense qu'il s'agit, non d'une structure, mais d'une propriété dynamique de la surface polarisée. Quoi qu'il en soit, la polarisation et l'imperméabilité relative sont liées et disparaissent ou s'atténuent ensemble.

La perturbation d'ailleurs n'est pas limitée à la surface; elle atteint la masse du protoplasma, comme on peut le voir en certains cas à l'ultra-microscope; la qualité optique paradoxale du protoplasma vivant, cette transparence parfaite d'une solution protidolipoïdique qui disparaît totalement à la mort, peut être légèrement, mais visiblement troublée d'une façon reversible par les excitations les plus diverses. La stimulation est un permier degré de lésion, comme l'aiguillon du bouvier (stimulus) est une lance minuscule.

Nous avons donc affaire à une perturbation qui affecte simultanément la polarisation, l'imperméabilité et l'homogénéité du protoplasma. Tel est le processus fondamental de l'excitation; il a pour chaque espèce de cellule son décours propre, lent ou rapide, que nous pouvons suivre en enregistrant le potentiel d'action; c'est lui que nous atteignons par la stimulation électrique (la cathode seule est efficace, parce qu'elle apporte les charges négatives neutralisant la polarisation positive), et c'est lui que concerne notre notion d'excitabilité, avec son caractère chronologique mesuré par la chronaxie.

Ceci nous explique le caractère uniforme, *homothétique*, de la loi d'excitation sur les objets les plus divers; dans des structures quelconques, nous atteignons toujours le protoplasma. La chronaxie est un paramètre protoplasmique. Nous verrons d'ailleurs plus loin que dans un tissu dont la chronaxie change par action pharmacodynamique, il y a modification des propriétés colloïdales, c'est-à-dire de la consistance du protoplasma.

Revenons à la comparaison de la contraction musculaire et du mouvement de la Sensitive. Celui-ci résulte d'une diminution de la turgescence du coussinet moteur; il y a sortie de liquide à travers les parois des cellules. C'est donc une conséquence immédiate de l'accroissement de perméabilité faisant partie du phénomène général d'excitation tel que nous venons de le voir.

Au contraire dans le muscle, la même perturbation protoplasmique déclanche une série de réactions chimiques; l'une de celles-ci, encore mal définie au milieu de la complexité des phénomènes, vient agir sur un colloïde distinct, la substance *isotrope* répartie au sein du protoplasma sous forme de fibres parallèles, avec ou sans disposition en chapelets (striation transversale); seule, cette substance aniso-trope est contractile au sens de la contractilité musculaire, c'est-à-dire avec force suivant un axe prédéterminé. Ce mécanisme chimico-colloïdal une fois déclanché a son allure propre en fonction du temps (forme de la contraction élémentaire examinée plus haut) et même, au moins dans les muscles striés, ses limites propres,

indépendantes de la grandeur comme de la forme du stimulus (loi du tout ou rien). Entre la durée de cette contraction, pour ainsi dire autonome, et les temps qui règlent l'efficacité du stimulus, l'expérience montre, comme nous avons vu, un certain accord nécessaire ; on n'agite pas un grelot comme on sonne une cloche de cathédrale ; mais évidemment accord différent du cas de la Sensitive.

Incidemment, notons que sous ce rapport, le pédicule de la Vorticelle se range à côté de la Sensitive et non parmi les muscles ; son mouvement est extrêmement rapide, d'une durée moindre que l'intervalle de deux images cinématographiques, c'est-à-dire moindre qu'un seizième de seconde (Belehradek). C'est au moins aussi rapide que la secousse du gastrocnémien de Grenouille ; or sa chronaxie est celle du cœur, près de dix fois plus grande que celle du gastrocnémien de Grenouille. Ceci est un argument contre la théorie qui voulait assimiler cet organe à un muscle, et en faveur de la conception opposée, à savoir une turgescence protoplasmique luttant contre un ressort, soit un fonctionnement analogue à celui de la Sensitive.

III. LA CHRONAXIE MUSCULAIRE ET LE CURARE.

Nous abordons maintenant un point de vue différent, mais qui peut recevoir beaucoup de lumière des faits et des considérations qui précèdent. La notion de chronaxie est née, comme nous avons vu, de la physiologie comparée ; quand elle présente quelque difficulté, il convient de la ramener à cette source. La controverse assez vive qui s'est développée récemment sur la signification physiologique de la chronaxie peut être tranchée net, si on veut bien l'élargir dans ce sens, au lieu de s'en tenir, comme faisait Du Bois-Reymond, à la sempiternelle Grenouille.

Toutes les expériences sur l'excitabilité rapportées plus haut, notamment toutes les mesures de chronaxie qu'elles comportent, ont été faites avec des électrodes fines constituées d'un fil métallique. C'est ainsi qu'opéraient Fick et Engelmann, et la généralité des auteurs classiques. D'autre fois, on s'est servi, en série avec un couple impolarisable, d'un pinceau effilé imbibé d'eau physiologique, ou même d'un fil de laine ou de coton imbibé de même. Dans ces conditions qui sont celles de la physiologie traditionnelle, on obtient, sauf une différence dans la rhéobase, la même courbe d'intensité-durée, la même chronaxie, dans la stimulation d'un muscle par son nerf et dans l'excitation directe de ce muscle.

Cette expression usuelle, excitation directe, signifie que les électrodes (tout au moins l'électrode active, la catode), sont posées sur le muscle. On est d'accord pour reconnaître que cela ne veut pas dire nécessairement que la substance musculaire est excitée elle-même par le stimulus électrique. Dans un muscle en général, il y a des fibres nerveuses qui se distribuent et se ramifient parmi les éléments musculaires. Le courant électrique diffuse d'une façon difficile à préciser dans cet entrelacement qui reste souvent indéterminé ; on ne sait pas alors si les fibres nerveuses ne sont pas mises en activité les premières. C'est la vieille querelle de l'irritabilité hallérienne qui remonte dans les siècles passés bien avant Galvani ; quand on pique, on coupe, on brûle un muscle fraîchement arraché de l'animal, il se contracte ; il est irritable, disait Haller. Mais la même violence portée sur son nerf produit les mêmes effets, ou plus grands encore ; la piqûre, la coupure, la brûlure atteignent

fatalement des filets nerveux intramusculaires; c'est, disait-on, par leur inter-médiaire que réagit le muscle, non irritable par lui-même.

La querelle fut tranchée quand Claude Bernard montra qu'un muscle empoisonné par le curare et qui ne réagit plus à aucune stimulation de son nerf est resté directe-ment excitable.

Claude Bernard, qui employait la stimulation électrique sous forme de pince de Pulvermacher (forme particulière de la pile de Volta), croyait même que l'excita-bilité musculaire restait sans changement. Mais E. von Brücke (1867) employant le chariot de Du Bois-Reymond constatait que le muscle curarisé, s'il reste à peu près aussi sensible aux *courants de pile*, c'est-à-dire à des courants prolongés, est devenu manifestement moins excitable pour les chocs d'induction, qui sont des courants brefs; d'où Brücke, par une application correcte des notions que Fick venait d'introduire dans la Science, conclut que le muscle curarisé présente une excitabilité plus lente. Il parut évident à Brücke que cette excitabilité lente était celle du muscle lui-même, tandis que l'excitabilité rapide présente avant curarisation était celle du nerf. Cette conclusion résultait directement des deux données posées par Claude Bernard et non contestées à cette époque: 1º le curare ne touche pas la substance musculaire; 2º son action porte sur l'*extrémité périphérique* des nerfs.

Cette théorie fut universellement admise, et je ne songeais pas à la mettre en doute au début de mes recherches sur la chronaxie. En effet, dès les premiers essais (1903), en employant la dose de curare juste suffisante pour paralyser le nerf, nous vîmes que le standard de temps qui alors nous tenait lieu de chronaxie était ainsi à peu près doublé.

Mais, avec des doses de curare plus fortes, nous vîmes des accroissements d'autant plus grands que la dose de curare était plus forte. Il n'y avait donc pas, comme l'implique la théorie classique, une chronaxie musculaire définie accessible après que le curare a mis les nerfs hors de cause.

Cette constatation me troubla; les essais divers que nous fîmes nous amenèrent de plus en plus à l'idée que le nerf et le muscle avaient normalement la même chronaxie, et que le curare *agissait sur le muscle* pour changer son excitabilité. Notamment, par cas fortuit, certaines positions d'électrodes nous donnaient une chronaxie augmentant déjà avant la paralysie du nerf, puis, avec des doses plus fortes que la dose curarisante, continuant à augmenter après cette paralysie: "La section physiologique se présente donc, disions-nous, non comme l'effet essentiel de l'intoxication, mais comme un effet accessoire" (1906).

Toutefois, nous n'osions encore affirmer explicitement ni l'isochronisme comme état normal entre le nerf et le muscle (deux éléments de structure si différente), ni l'hétérochronisme comme mécanisme de la curarisation (audacieuse hérésie vis-à-vis des dogmes classiques!).

Nous cherchâmes des contre-épreuves. Nous avions évidemment pensé à utiliser, pour la vérification directe de l'isochronisme, la disposition bien connue du muscle couturier, qui offre une partie dépourvue d'éléments nerveux, mais l'excitabilité de ce muscle une fois disséqué, se révèla instable, disparaissant au bout d'un temps assez court, après une montée continuelle de la chronaxie.

Au contraire, l'examen de divers poisons curarisants se montra très instructive; on a signalé en effet depuis longtemps d'autres substances qui, comme le curare, ne suppriment pas l'excitabilité indirecte quand on les applique sur le nerf seul, mais qui la suppriment en respectant l'excitabilité directe quand on les applique sur le muscle (épreuve de Claude Bernard).

Tel est le cas de la strychnine (Vulpian). Or, en étudiant ce cas, Mme Lapicque (1907) obtint le double résultat suivant: 1° après disparition de l'excitabilité indirecte, le muscle présente encore la même chronaxie qu'à l'état normal; donc la théorie classique ne peut s'appliquer à la curarisation par la strychnine; 2° mais pendant que la curarisation s'installe, le nerf, tant qu'on peut le suivre, présente une chronaxie constamment en diminution, arrivant à peu près à la moitié de sa valeur primitive au moment où l'excitabilité indirecte disparaît. Nous avons donc encore un hétérochronisme, différent de celui du curare, et néanmoins concomitant aussi de l'arrêt de la transmission nerf-muscle.

Quelques mois après, nos dernières hésitations furent levées, quand, au Congrès international de Physiologie à Heidelberg, nous entendîmes Langley (1908), s'appuyant sur une série de belles recherches totalement différentes des nôtres, affirmer que le curare n'agit pas sur les terminaisons nerveuses, mais sur une partie intégrante de la substance musculaire. Il me parut nécessaire d'expliciter tout de suite l'accord de nos résultats avec ceux de Langley et de donner, séance tenante, une esquisse de notre théorie chronologique de la curarisation.

La théorie classique supposait une action du curare portant exclusivement sur les terminaisons nerveuses. Mais une telle localisation pharmacodynamique, indépendamment de son incapacité à expliquer nos résultats, se heurte à des objections d'ordre général qui venaient d'être mis en lumière à l'occasion d'une série de recherches des Physiologistes de Cambridge. Je ne puis entrer ici dans le détail de ces recherches, dont j'ai récemment résumé l'historique ailleurs (L. Lapicque, 1934 *b*); il s'agissait primitivement du mode d'action des substances appelées aujourd'hui sympathomimétiques et parasympathomimétiques. Dixon, Brodie, Elliott, Anderson tendaient à localiser cette action sur les *terminaisons nerveuses*, modifiées en *jonctions myoneurales*. Mais, dit Langley (1905), il n'existe aucune structure anatomique distincte entre le nerf et le muscle; et quelque chose qui serait partie intégrante à la fois du nerf et du muscle est impensable au point de vue de l'anatomie générale.

Je regrette de n'avoir pas pensé à m'appuyer sur ces fermes considérations de Langley quand j'ai repris de mon côté (1926, p. 271) la critique des prétendues *plaques motrices*, siège de l'action du curare. Langley mène sa discussion d'un point de vue général, disant: une substance n'agissant pas sur le nerf ne peut agir qu'au delà du nerf, c'est-à-dire sur le muscle, et il réalise une série d'expériences montrant que tel est bien le cas du curare.

Des expériences postérieures ont confirmé pleinement cette localisation du curare.

1° Un muscle baigné dans du Ringer légèrement hypotonique s'imbibe suivant une courbe définie qu'on peut suivre en le pesant d'heure en heure. Après curarisation, cette imbibition est moindre, s'effectuant plus lentement et atteignant un maximum moins élevé; la différence est considérable, se chiffrant par une dizaine

de centigramme pour un muscle d'environ un gramme (L. et M. Lapicque, 1914). Quelque soit le mécanisme physicochimique qui constitue la base de ce changement d'hydrophilie, il ne peut concerner exclusivement les terminaisons nerveuses, de quelque façon qu'on comprenne celles-ci; seul le muscle possède une masse suffisante pour rendre compte de la capacité d'absorption ainsi modifiée.

2° Dans un muscle curarisé, il y a modification quantitative au moins de l'une des réactions chimiques qui font partie du phénomène normal de la contraction. Les mutations du *phosphagène* sont diminuées et cette diminution varie avec le degré de curarisation comme l'augmentation plus ou moins grande de la chronaxie que nous avons indiquée et que les auteurs ont vérifiée (Meyerhof, 1929; Nachmansohn, 1929).

Donc le curare est bien, comme Langley l'a publié le premier, un poison musculaire.

La contraction du muscle n'est pas changée; sans doute! Si le curare ralentissait la contractilité comme il ralentit l'excitabilité, son mode d'action serait apparu clairement depuis Claude Bernard. La physiologie classique a raison de proclamer unanimement que la substance contractile n'est pas touchée par le curare; où elle a fait erreur, c'est quand elle a conclu de là que rien n'est changé dans le muscle, confondant implicitement la substance contractile avec le muscle tout entier. Pourtant, il y a une distinction bien nette et non moins classique, entre la substance anisotrope et la substance isotrope ou protoplasma. Nous avons vu plus haut qu'il y a des raisons d'attribuer à ce dernier l'excitabilité, tandis que la contractilité appartient à la première. Dès lors, on comprend qu'une action pharmacodynamique puisse changer une des fonctions et non l'autre.

Mais alors, que devient la liaison sur laquelle nous avons insisté entre la vitesse de contraction et la vitesse d'excitabilité? Nous avons vu aussi que cette liaison est indirecte, et d'autre part qu'il ne s'agit pas d'une proportionnalité stricte. Le gastrocnémien a une contraction environ 400 fois plus rapide que celle de l'estomac, et le rapport des chronaxies est 1 à 2000. Les différences produites par le curare sont petites relativement à cette série; d'ailleurs il semble bien que la perturbation du rapport normal, si elle n'empêche pas le processus de s'accomplir à l'intérieur du muscle comme elle l'empêche entre le nerf et le muscle, y apporte néanmoins quelque difficulté; le curare en effet augmente la rhéobase musculaire, c'est-à-dire exige une stimulation plus intense. On saisit précisément quelque chose de ce genre pour l'excitation indirecte, dans les premiers stades de la curarisation; pendant que l'hétérochronisme s'établit progressivement jusqu'au degré où il s'arrête totalement la transmission, la rhéobase de la stimulation nerveuse s'élève *pari passu* progressivement jusqu'à l'infini.

IV. L'ISOCHRONISME NEURO-MUSCULAIRE: RÉSULTAT UNIVOQUE DE SES DIVERSES PERTURBATIONS.

L'hétérochronisme entre le nerf et le muscle a toujours cet effet, quelle que soit la cause qui l'ait produit et dans quelque sens qu'il se produise. Les observations de ce genre peuvent être décrites objectivement sans postuler l'isochronisme;

puisque sous la seule condition d'opérer avec des électrodes fines et pas trop rapprochées, la stimulation directe et la stimulation indirecte donnent sur le muscle normal approximativement la même chronaxie, partons de cette égalité de fait.

J'ai étudié ou fait étudier systématiquement un certain nombre de poisons classés comme curarisants (Joteyko, 1900). Curariser veut dire, suivant la généralisation usuelle, supprimer la transmission de l'excitation entre un nerf excitable et un muscle excitable. Nous avons toujours commencé par faire subir à chaque poison l'épreuve de Claude Bernard ; quelques-uns ont supprimé l'excitabilité indirecte quand on les appliquait sur le nerf seul ; ce n'étaient donc pas réellement des curarisants, mais simplement des poisons du nerf. Tous les autres ont modifié la chronaxie, soit du muscle seul, soit du nerf seul, soit des deux, mais alors dans des sens opposés. Quand les deux chronaxies, au lieu d'être dans un rapport voisin de l'unité, diffèrent comme de 1 à 2 ou à 3, l'excitabilité indirecte disparaît.

Nous avons déjà vu le curare et la strychnine. La spartéine augmente la chronaxie directe, curarise donc comme le curare ; il en est de même des amines, notamment des amines quaternaires, souvent rapprochées du curare dans les classifications pharmacodynamiques, et parmi lesquelles on a même voulu englober la substance active du curare ; toutefois, ces amines présentent d'abord une phase passagère de diminution de chronaxie, tant directe qu'indirecte, phase que je n'ai jamais observée avec aucun curare.

La strychnine, à ma connaissance, est la seule substance qui curarise en diminuant la chronaxie nerveuse. Remarquons que dans ce cas, avec un mécanisme différent, l'hétérochronisme est de même sens que dans le cas précédent.

Au contraire, la vératrine, la nicotine, la physostigmine, la pilocarpine et diverses autres substances, dans la première phase de leur action, laissant à sa valeur primitive la chronaxie indirecte après une diminution passagère, diminuent la chronaxie directe ; si celle-ci descend à la moitié de sa valeur primitive ou un peu au-dessous, ce qui n'arrive pas toujours, l'excitabilité indirecte disparaît. C'est alors une curarisation par hétérochronisme inverse des cas précédents. Ces mêmes poisons, à un degré d'intoxication plus avancé, c'est-à-dire avec une dose plus forte et un temps d'action plus prolongé, présentent une deuxième phase où la chronaxie directe s'accroît au double et davantage de la valeur primitive ; l'excitabilité indirecte disparaît alors, qu'elle se soit ou non maintenue pendant la première phase ; nous avons de nouveau une curarisation *type curare.*

Avec cette dernière catégorie de poisons, l'évolution totale de la chronaxie directe comporte évidemment, après la phase de diminution, une remontée passant par la valeur normale pour arriver à l'augmentation de la seconde phase ; cette remontée est rapide ; néanmoins on a pu quelquefois saisir, au cours de l'intoxication d'un même muscle, 1° une curarisation avec chronaxie directe diminuée ; 2° un court rétablissement spontané de la transmission ; 3° une curarisation avec chronaxie directe augmentée (M. Lapicque, 1922).

Tous ces faits variés peuvent se résumer dans la formule suivante : les actions pharmacodynamiques curarisantes produisent un hétérochronisme en même temps qu'elles suppriment l'excitabilité indirecte. Cet hétérochronisme se manifestant

sous trois formes différentes, je ne vois pas comment on pourrait maintenir la donnée classique qu'il résulte de la suppression de l'excitabilité indirecte.

On a dit, en considérant le seul curare, que l'hétérochronisme pouvait être un phénomène surajouté, sans lien avec la curarisation proprement dite, et même causé par un composant de la drogue autre que le principe curarisant. L'idée d'une telle coïncidence fortuite ne peut être maintenue devant la multiplicité et la variété des cas dont je viens de donner quelques exemples parmi beaucoup d'autres. Bien plus, quand l'hétérochronisme se produit par une cause quelconque autre que les poisons, l'arrêt de la transmission se produit tout aussi bien, et peut légitimement, en étendant encore le terme, être qualifié curarisation.

Par exemple, quand un muscle comme le couturier, plus généralement un muscle *atonique*, augmente spontanément de chronaxie après sa dissection, il cesse bientôt de répondre à la stimulation de son nerf (L. et M. Lapicque, 1932).

Un cas plus important physiologiquement est celui de la fatigue; on sait depuis longtemps qu'un muscle soumis par stimulation indirecte à un travail prolongé jusqu'à ce qu'il cesse de répondre est encore capable de répondre à la stimulation directe et de donner pendant longtemps de belles contractions. Ce phénomène a été rapproché avec raison de la curarisation, mais on l'expliquait, conformément à la théorie en vogue, par la fatigue des terminaisons nerveuses. Or la chronaxie directe augmente progressivement pendant le travail fatigant, et elle est sensiblement doublée au moment que la stimulation indirecte cesse d'être efficace (L. et M. Lapicque, 1919).

La seule hypothèse simple et générale, capable d'interpréter cette série de faits, est évidemment de considérer l'hétérochronisme comme la cause de la curarisation. Les antagonismes des poisons confirment cette hypothèse.

Rothberger (1901 et 1902) a signalé certains poisons capables de supprimer la curarisation (par le curare); j'ai constaté que tous ont comme action propre de diminuer la chronaxie musculaire; ils peuvent même curariser de cette façon. Quand on les fait agir après curare, ou réciproquement, on constate que les actions inverses des deux substances s'additionnent algébriquement; la chronaxie altérée par le premier poison revient vers la normale sous l'effet du second. Autrement dit, c'est en rétablissant l'isochronisme que les antidotes du curare suppriment la curarisation.

Plus significatif encore est un autre antagonisme, celui de la strychnine contre la vératrine ou la pilocarpine, c'est-à-dire contre la curarisation par diminution de chronaxie musculaire.

Dans le cas précédent, l'antidote agissait sur le même élément physiologique que le poison pour contrecarrer son action; ici, l'antidote agit sur l'autre élément du couple neuro-musculaire pour y produire une action de même sens que le poison; ainsi l'isochronisme peut être rétabli entre les éléments altérés tous deux.

Cette expérience, conçue théoriquement quand nous eûmes analysé l'action de la strychnine d'une part, l'action de la vératrine d'autre part, nous paraissait très risquée; même si l'isochronisme est la condition nécessaire pour le passage de l'excitation d'une cellule à une autre, ce n'est évidemment pas une condition suffisante; notamment, il n'en résulte pas *à priori* qu'un muscle empoisonné doive

réagir sous l'action d'un nerf également empoisonné redevenu isochrone, car l'intoxication a pu altérer dans l'un ou l'autre élément bien autre chose que la chronaxie. Mais si elle réussissait l'expérience était singulièrement suggestive. Elle a réussi (L. et M. Lapicque, 1912).

Les antagonismes dans la curarisation me paraissent avoir la valeur d'une preuve cruciale transformant l'hypothèse en théorie démontrée.

Si la curarisation est produite par n'importe quel hétérochronisme, la nécessité de l'isochronisme comme état normal en découle. C'est par cette voie que nous avons été amenés à le concevoir ; nos expériences nous indiquaient d'ailleurs que la chronaxie directe, sur le muscle normal, peut être aussi bien celle de la substance musculaire que celle des nerfs ; par exemple, la variation continue de la chronaxie au cours de l'intoxication, commençant parfois à monter ou à descendre depuis l'état normal, sans point singulier au moment où l'excitabilité indirecte disparaît ; à partir de ce moment, il est bien sûr que l'excitabilité observée est celle de la substance musculaire ; la chronaxie préalable, sensiblement égale à celle du nerf, se rattachant par une évolution continue à la chronaxie du muscle curarisé, appartient donc à la même substance ; dans d'autres cas, la chronaxie directe se maintient constante un certain temps, puis monte brusquement à une valeur double ou triple, indiquant que nous venons de passer de l'excitabilité nerveuse à l'excitabilité musculaire ; la chronaxie de cette dernière continue à augmenter s'il s'agit de curare ou de spartéine à dose un peu forte ; cette évolution, extrapolée jusqu'à l'origine, conduit sensiblement à l'égalité avec la chronaxie nerveuse.

Pour la strychnine, le cas est encore plus net, puisque la chronaxie de la stimulation directe reste la même après disparition de l'excitabilité indirecte ; que serait cette chronaxie, si elle n'était celle du muscle lui-même ?

Néanmoins, nous avons en 1925 cherché une nouvelle preuve en faisant appel à la considération suivante, fort importante d'ailleurs par elle-même pour la théorie chronologique de la transmission.

Le courant électrique a son efficacité diminuée ou même abolie quand, au lieu de s'établir brusquement, il s'établit progressivement. Ce fait connu depuis le début du XIXe siècle (Ritter), a été pour Du Bois-Reymond une base essentielle de sa fameuse loi. Mais la brusquerie nécessaire n'est pas absolue ; elle est relative à la vitesse propre de l'excitabilité examinée ; tandis que, sur la Grenouille, quelques centièmes de seconde employés à l'établissement du courant suffisent à le rendre inefficace, Fick a vu sur l'Anodonte qu'un retard d'une dizaine de secondes était sans influence et qu'il fallait porter le retard à 120 secondes pour supprimer tout effet. Divers auteurs, dont Keith Lucas, ont vérifié cette loi sur divers objets. Il s'agit d'un phénomène qui rentre encore dans la notion de chronaxie ; on devra, je crois, rectifier la règle de simple proportionnalité que j'avais donnée en première approximation, mais il est sûr que le retard à l'établissement du courant a d'autant plus d'importance que la chronaxie est plus petite.

L'application de cette loi permet, dans un complexe de deux excitabilités, de mettre en jeu sélectivement la plus lente même quand celle-ci a la rhéobase la plus élevée, comme c'est généralement le cas. Dès 1908, nous avions réussi à démontrer

expérimentalement cette excitation sélective sur deux muscles, l'un de Grenouille, l'autre de Crapaud, disposés dans le même circuit.

En 1925, nous avons songé à appliquer cette méthode au complexe nerf-muscle au cours de la curarisation; on avait soin que les électrodes restassent exactement sur le même point du muscle; le retard à l'établissement était gradué par des condensateurs en dérivation.

Voici des chiffres extraits de nos expériences (Gastrocnémien de Grenouille). Avant curarisation, le voltage liminaire passe de o·60 V. pour le courant brusque à 1·60 V. pour un retard de 6 centièmes de seconde, soit de 1 à 2·7; après curarisation, le voltage liminaire passe de o·70 V. pour le courant brusque à 1·10 V. pour le même retard, soit de 1 à 1·6.

Cette moindre influence du retard caractérise une excitabilité plus lente dans le second cas que dans le premier; c'est sous une autre forme l'augmentation de chronaxie bien connue; mais de plus ici, on peut reconnaître que cette excitabilité lente, qui appartient certainement au muscle, ne préexistait pas dans le complexe normal. En effet, le seuil pour le courant retardé est, en valeur absolue, plus bas après curarisation; peu importe que le seuil antérieur ait concerné le nerf ou le muscle; un seuil bas ne peut être masqué par un seuil plus haut; il ne s'agit donc pas d'une excitabilité musculaire normalement lente démasquée par la suppression de l'excitabilité nerveuse, mais d'une lenteur créée par le curare sur le muscle, qui était pratiquement isochrone au nerf à l'état normal.

V. RÉPONSE AUX OBJECTIONS: FAUSSES CHRONAXIES.

A ce même Congrès de Bruxelles en 1907, où nous avions eu le plaisir de constater notre accord avec Langley, Keith Lucas apportait des expériences où apparaissaient, par la stimulation directe du couturier, 3 excitabilités distinctes quant à leur caractère chronologique; sans parler de la 3e, qui n'a pas été reprise en discussion, Keith Lucas appelait la plus rapide γ, et l'attribuait à la substance nerveuse, car la stimulation du sciatique (avec la contraction du gastrocnémien comme test), donnait à peu près la même; il appelait la plus lente α, et l'attribuait à la substance musculaire, parce qu'elle seule était donnée par la partie aneurale du muscle.

Le raisonnement était judicieux, et la conclusion en plein accord avec la doctrine de Brücke classique depuis 40 ans sans contestation; la différence de α à γ semblait une mesure de l'hétérochronisme neuro-musculaire postulé par cette doctrine. Par cela même, elle était inconciliable avec notre conception, que justement Langley venait de confirmer indirectement, et d'autre part, nous n'avions jamais, sur les muscles les plus divers, rien observé de pareil.

Il faut noter qu'à cette époque, où le mot *chronaxie* n'existait pas, où je n'avais pas non plus dégagé la notion de rhéobase, nous ne recourions pas à la mesure simplifiée devenue classique et qui est liée à la définition pratique de la chronaxie. Dans chaque expérience, nous faisions un certain nombre de déterminations nous permettant de tracer la courbe intensité-durée, d'où nous déduisions un paramètre chronologique à la fois graphiquement et algébriquement. Avec cette façon d'opérer,

qui paraissait identique à celle de Keith Lucas, pourquoi n'avions-nous jamais, au cours de 5 années de recherches, rencontré comme lui la double excitabilité du nerf et du muscle ? Je posai amicalement cette question à Keith Lucas, qui fut, aussi bien que moi, incapable d'assigner une cause à nos divergences.

Cette cause n'a été reconnue que très récemment, après que Rushton (1930), stimulant le couturier par des courants électriques diffus dans un bain de Ringer, eut retrouvé deux courbes d'excitation incontestablement distinctes. Dans les expériences de Keith Lucas, la différence était souvent peu nette, et je l'avais considérée comme illusoire ; aujourd'hui, je rends hommage à la pénétration avec laquelle Keith Lucas avait su la saisir. Dans les expériences de Rushton, avec un γ qui est toujours à peu près le même, il y a un α considérablement plus lent. Dès lors, il m'apparut (1931) que la production d'une courbe α était imputable à la stimulation dans un bain électrolytique, α étant plus lent pour Rushton que pour Keith Lucas parce que le bain était plus ample. En effet, Keith Lucas avait cherché à éviter l'altération du couturier en le mettant à l'abri de la dessiccation ; pour cela, il avait imaginé ses électrodes fluides, où le muscle est suspendu dans une atmosphère close entre deux bains de Ringer ; le tube supérieur en contient une longueur de quelques millimètres dans le liquide formant catode. La longueur de cette partie conditionne la courbe α, comme Matton (1932) l'a démontré. Dans le dispositif de Rushton, le muscle tout entier était dans le liquide siège du champ électrique stimulant ; en rétrécissant la longueur suivant laquelle le muscle est soumis à ce champ, on fait varier la courbe α à volonté (L. Lapicque, 1931).

D'autre part, le plus souvent, cette courbe s'écarte notablement de la courbe générale de notre Fig. 3 ; elle correspond donc à un processus de stimulation autre que celui des électrodes fines et la valeur d'abscisse pour laquelle elle passe par une hauteur double de sa hauteur pour les temps très longs n'est pas homogène à une chronaxie ; cette raison m'a suffi, dès le début de la discussion, pour annoncer qu'il s'agissait d'une pseudo-chronaxie. Ce raisonnement était l'application pure et simple d'un principe élémentaire. Mais Rushton tourna en dérision ma prétention à distinguer des vraies chronaxies et des fausses chronaxies, et m'accordant ironiquement, comme inventeur du mot, le droit de le définir selon ma fantaisie, il proposa, pour échapper à mon arbitraire, d'y substituer l'expression "temps d'excitation" ="excitation-time" employé par Keith Lucas. Celui-ci s'est servi provisoirement de cette expression, pendant qu'il cherchait, comme moi, une constante de temps rationnelle ; je ne pense pas qu'il l'aurait acceptée comme terminologie formelle. Il est impossible de poser clairement une discussion sur un terme aussi ambigu ; il y a pour un tissu donné dans des conditions données une infinité de "temps d'excitation" suivant l'intensité du stimulus. Ce qu'on veut dire, c'est "excitation time for 2 rheobases". Disons-le explicitement, en abrégeant au moyen des initiales : ET2R. Le temps ainsi mesuré sera alors, conformément au desideratum de Rushton, une mesure sans signification *à priori*, permettant de comparer chronologiquement des courbes intensité-durée de formes grossièrement semblables pouvant traduire des processus divers. Si on s'en tient là, évidemment Rushton a raison de dire que cette mesure est peu intéressante. Mais parmi les ET2R très divers que peut

fournir un muscle stimulé par des électrodes de grandeur quelconque, il y a une valeur et une seule, qui possède une signification physiologique.

Davis (1923) a affirmé nettement, mais sans détails expérimentaux que la chronaxie d'un muscle varie systématiquement avec la grosseur des électrodes; Watts (1924) a confirmé le fait, avec des précisions erronées. J'ai repris (1932) la question en employant comme électrode active des tubes de verre de diamètres différents remplis de Ringer, et appliqués normalement à la surface du muscle; il est apparu alors une loi bien nette: la Fig. 4 représente graphiquement pour 2 expériences sur le couturier de la Grenouille, ET2R en fonction du diamètre de la catode. Du côté des grands diamètres, cette courbe rejoint celle des longueurs
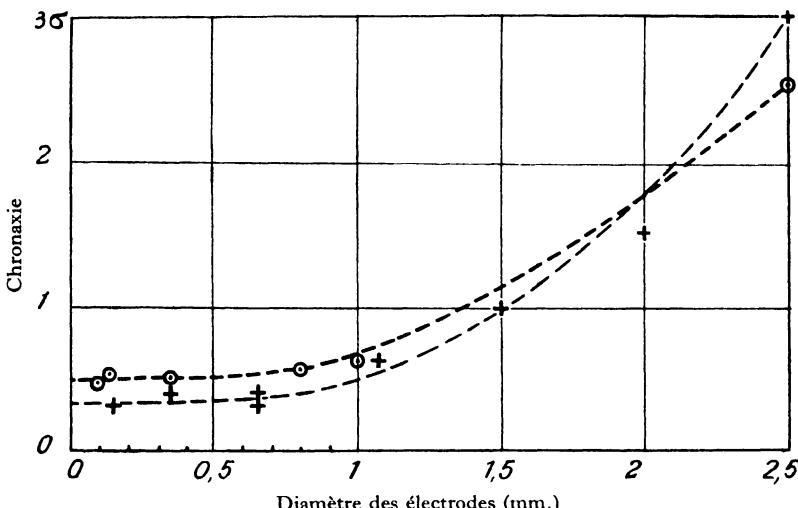

Fig. 4. Deux expériences sur le couturier de la Grenouille avec des électrodes de diamètre croissant; la chronaxie, invariable jusqu'à 0·6 ou 0·7 mm., fait place ensuite à une fausse chronaxie d'autant plus grande que l'électrode est plus grosse.

de muscle soumises au courant dans les bains suivant la méthode de Rushton, et là nous ne trouvons qu'une limite supérieure assez vague, 10 à 20σ. Mais du côté des petits diamètres la courbe tend vers une valeur constante voisine de 0·3 σ atteinte pratiquement à 6 ou 7 dixièmes de millimètre, c'est-à-dire qu'au-dessous de, disons, un demi-millimètre, ET2R est indépendant de la grosseur de l'électrode.

Nous avons là une grandeur définie, non seulement une limite théorique, mais une condition pratique de mesure stable. Nous sommes revenus ainsi au cas des électrodes fines, qui, après avoir permis de constituer physiologiquement la notion de chronaxie, ont conduit à l'isochronisme neuro-musculaire et à la théorie chronologique de la curarisation. Si au contraire, on veut, avec Rushton, considérer l'ET2R donné par les larges électrodes comme représentant l'excitabilité musculaire, où prendrons-nous cette valeur caractéristique? Rushton ne donne explicitement aucun chiffre pour la "chronaxie" de sa substance α; ses courbes indiquent

des valeurs de 7 à 17σ (Keith Lucas avait trouvé de 3 à 8σ). Quand j'eus montré (1931) l'influence prépondérante de la dimension du bain électrolytique, il abonda dans ce sens (1932 *a*, p. 185), puis en vint à proclamer que l'"excitation time" dépend toujours à la fois du tissu et des conditions physiques de la stimulation (1933, p. 360); alors l'isochronisme ou l'hétérochronisme résulte du choix qu'on fait de ces conditions physiques, pratiquement, pour le muscle, de la grosseur de la catode. Rushton en convient explicitement; mais après avoir, en débutant, affirmé l'hétérochronisme en s'appuyant sans réserve sur l'emploi de larges électrodes, il est resté partisan de cette méthode sans avoir une bonne raison à en donner; en 1932 (*b*) il a esquissé une théorie physique d'où résulterait que les petites électrodes altèrent une excitabilité α normalement grande pour en faire une apparence d'excitabilité rapide, "γ-like"; mais, ajoute-t-il prudemment, on ne sait pas si le phénomène invoqué est d'un ordre de grandeur convenable. Dans son récent article (1935, p. 5), il va plus loin, et déclare que le choix à faire est complètement arbitraire; l'idée d'un ET2R plus correct qu'un autre, dit-il, n'a pas de sens physique. Nous reviendrons tout-à-l'heure sur le point de vue physique, mais je vais montrer d'abord qu'au point de vue physiologique, le choix s'impose.

Il suffit, pour être fixé là-dessus, de retourner à l'origine de la chronaxie, et à la physiologie comparée qui lui a donné naissance. Nous avons vu comment ET2R varie avec la grosseur des électrodes sur un muscle rapide de Grenouille; faisons sur un muscle de Tortue (durée de contraction 1·5 à 2 secondes, soit 10 fois plus grande que pour la Grenouille), la même expérience, tant avec la série des électrodes capillaires qu'avec des bains à la Rushton plus ou moins amples. On obtient une courbe de forme analogue. Il y a pour les électrodes les plus fines, une limite inférieure à environ 2σ, soit presque 10 fois plus grande que chez la Grenouille; pour les bains les plus amples, on obtient des ET2R de 10 à 20σ, soit les chiffres mêmes que j'ai trouvés sur la Grenouille (1932) en accord avec ceux de Rushton. Les électrodes les plus larges donnent toujours les mêmes valeurs, par exemple sur le muscle longitudinal de l'Holothurie, encore plus lent (durée de contraction, 6 à 8 secondes), les électrodes les plus petites donnant ici un ET2R de 5 à 10σ. De sorte que, sur 3 muscles dont les durées de contraction sont respectivement comme 1, 10 et 40, les petites électrodes donnent des ET2R qui sont entre eux sensiblement dans la même proportion que ces durées; ce sont donc des chronaxies. Les grandes électrodes donnent pratiquement le même ET2R pour les muscles lents et les muscles rapides. Ce ne sont pas du tout des chronaxies, si on veut conserver à ce terme un sens physiologique.

Cette conclusion est corroborée par l'influence des variations de température. Il n'y a pas de doute qu'un muscle refroidi est ralenti dans tous ses processus. ET2R mesuré avec des électrodes fines (c'est-à-dire la chronaxie) indique bien ce ralentissement; elle double à peu près pour un abaissement de température de 10°. Mais avec les plus grandes électrodes, ET2R n'augmente pas du tout ou même diminue un peu (Benoit, 1933).

Après cela, on jugera, j'espère, que Rushton est injuste quand il m'accuse (1935, p. 5) de déclarer correctes les petites électrodes uniquement parce qu'elles

donnent des résultats conformes à ma théorie de l'isochronisme; l'argument ci-dessus avait été publié sommairement en Français en 1932, très explicitement dans le *Journal of Physiology* en 1933, et rappelé comme point de départ pour la discussion dans ce même *Journal* en 1934.

Donc, il est démontré que seules les petites électrodes permettent de saisir dans le processus de l'excitation une caractéristique chronologique des muscles. Néanmoins, on ne peut éviter la question: comment se fait-il que la grosseur des électrodes influe fortement sur le temps impliqué dans ce processus? Rushton (1935, p. 7), persistant à m'accuser de cercle vicieux, déclare humoristiquement que la solution de ce problème varie suivant qu'on l'aborde en physicien ou en isochroniste. Je viens de discuter d'abord, comme il convenait, en physiologiste; discutons sur le terrain physique.

Nous sommes d'accord pour prendre comme point de départ la conception de Nernst, qui est la seule explication précise du mécanisme physicochimique de l'excitation électrique. La cause prochaine de l'excitation est la polarisation que le courant électrique produit sur les interfaces cellulaires; pour le calcul quantitatif, on considère schématiquement un conducteur électrolytique cylindrique coupé par une cloison semiperméable perpendiculaire aux génératrices; le courant accumule contre cette paroi certains ions (ou certains sels); la diffusion tend continuellement à disperser cette accumulation; pour le courant constant, la somme algébrique ainsi calculée croît proportionnellement à la racine carrée du temps. Ce résultat de mathématiques assez ardues peut être retrouvé intuitivement sur des modèles matériels simples; par exemple, la croissance en hauteur d'un tas de sable projeté à pelletées régulières contre un mur vertical, entre deux murettes perpendiculaires à celui-là. Le dispositif d'électrodes qui m'a permis de préciser la relation entre leur diamètre et l'ET2R reproduit aussi exactement que possible les conditions postulées par Nernst. La théorie de Rushton s'y applique clairement. La densité du courant pénétrant dans le muscle est supposée homogène, comme dans le schéma de Nernst, mais outre la diffusion en sens inverse, qui est la seule à contrecarrer la polarisation dans ledit schéma, il y a, sous les bords de l'électrode, diffusion latérale. Cette diffusion latérale, dit Rushton, prend une importance d'autant plus grande que l'électrode est plus petite; or le décrément supplémentaire qu'elle apporte au développement de la polarisation limite le temps disponible pour le processus de l'excitation; par conséquent, la diminution du calibre de l'électrode introduit un phénomène purement physique qui fait paraître artificiellement des temps d'excitation courts.

Je suis pleinement d'accord sur l'existence de ce phénomène, et sur le sens dans lequel il agirait. Mais est-il d'un ordre de grandeur suffisant pour intervenir effectivement dans nos résultats? Rushton a eu tort d'oublier cette réserve qu'il formulait en 1932.

Nous avons deux façons d'apprécier cet ordre de grandeur; 1° il est évidemment le même, en fonction du temps et de l'espace, pour cette diffusion latérale et pour la diffusion en arrière indiquée par Nernst. Dans notre modèle de sable, supprimons les murettes latérales; le sable s'éboule sur les côtés avec la même pente qu'en

s'éloignant du mur. Or le calcul de Nernst, qui se vérifie expérimentalement dans une mesure importante, suppose que la diffusion en arrière est petite par rapport aux dimensions cellulaires ; cela nous limite à des longueurs de l'ordre du millième de millimètre. 2° La considération des grandeurs et des distances des ions ou des molécules accumulés et diffusant nous conduirait à des dimensions bien au-dessous de cette limite. Rushton a donné une image où ces particules sont figurées par des personnes débouchant d'une rue sur une vaste place ; suivons cette comparaison : il faudrait que la rue ait plusieurs kilomètres de large pour correspondre à mes petites électrodes. D'une façon comme de l'autre, on voit que la diffusion latérale ne peut affecter qu'une mince frange le long du bord. Cette perturbation marginale n'intervient pas du tout dans nos mesures d'excitabilité ; en effet, la polarisation, cause de l'excitation, n'y atteignant pas le même niveau qu'ailleurs, c'est la région centrale non affectée de la perturbation qui conditionne l'excitation. On ne voit donc pas comment la physique permettrait d'attribuer une cause d'erreur théorique aux électrodes fines qui donnent pratiquement les résultats physiologiquement corrects.

Quant aux électrodes larges, j'ai, dès 1932, indiqué le principe d'une théorie physique expliquant l'erreur qu'elles introduisent dans les mesures. Le phénomène fondamental de cette théorie est que la polarisation, au lieu de se développer uniformément, comme le suppose la loi de Nernst et comme cela se passe pratiquement dans les petites électrodes, commence sur un bord, du côté de l'anode, mais se déplace avant d'atteindre le niveau où elle crée l'excitation ; elle recule ainsi jusqu'au bord opposé, où le processus s'achève enfin. Rushton a interprété d'une manière entièrement inexacte l'exposé pourtant explicite que j'en avais donné, et critiqué ma théorie en se fondant sur cette interprétation (1933, p. 360). Cette critique tombe entièrement à faux. La *polarisation rétrograde* est actuellement approfondie au point de vue physique dans mon laboratoire par A. M. Monnier et P. Benoit ; elle suit bien la marche que je lui avais assignée *à priori* et semble devoir rendre compte en détail des résultats physiologiques.

Le choix des électrodes à employer dans l'étude chronaximétrique étant ainsi déterminé pour des raisons à la fois expérimentales et théoriques, tous les arguments que j'ai donnés plus haut en faveur de l'isochronisme neuromusculaire, arguments fondés sur l'emploi des électrodes fines, sont validés.

Nous pouvons maintenant y ajouter une constatation directe. En effet, le couturier, jadis rebelle aux mesures, s'y prête assez docilement depuis que Dulière et Horton (1929) ont expliqué le mécanisme de ses altérations et indiqué le moyen de les éviter. Il suffit d'opérer dans un bain de Ringer. C'est ce qu'avait fait empiriquement Keith Lucas avec ses électrodes fluides ; mais on peut, dans ce bain, limiter la catode aux dimensions requises en la constituant par un fin tube de verre ; on obtient alors, sur la partie aneurale du muscle, c'est-à-dire sur une excitabilité incontestablement musculaire, une chronaxie régulièrement voisine de 0·3 σ, ce qui est le chiffre admis sans conteste pour la chronaxie nerveuse du membre de la Grenouille. La comparaison, non plus entre des moyennes, mais individuellement sur un muscle donné, avec son propre nerf, est difficile chez la Grenouille, car le

petit rameau nerveux du couturier, une fois isolé, est très fragile; mais nous avons couramment réussi l'expérience avec le grand et robuste Crapaud de l'Afrique du Nord, *Bufo pantherinus*. On trouve, pour le nerf et pour la partie aneurale du muscle des valeurs parfois égales, toujours très voisines (L. Lapicque, 1934 *a*).

L'isochronisme neuromusculaire devient ainsi un simple fait d'expérience.

VI. SOLUTION DES LITIGES SECONDAIRES.

Il me semble que Rushton n'est plus très loin d'abandonner son opposition. Son dernier article, celui qui a paru dans ce journal au début de la présente année, a été encore écrit dans un sens entièrement négatif, mais il a intercalé (p. 13) trente lignes en petit texte me faisant des concessions peu explicites, mais au fond assez importantes pour ébranler toutes ses conclusions négatives.

Rushton n'admettait pas comme démontré que le curare augmente la chronaxie musculaire (les électrodes larges ne lui ont pas donné cette augmentation; mais comment la donneraient-elles, puisqu'elles ne marquent pas la différence entre les muscles rapides et les muscles lents?) ni que la marche continue de la chronaxie au cours d'une curarisation indique une chronaxie petite avant la paralysie du nerf. Il reconnaît que mes raisons expérimentales d'affirmer cette augmentation de chronaxie étaient excellentes.

Rushton avait déclaré que l'antagonisme vératrine-strychnine n'existe pas; j'avais expliqué (1934 *a*) ses expériences négatives en admettant qu'elles avaient porté sur la seconde phase de la curarisation vératrinique, où il y a augmentation de chronaxie musculaire; la strychnine diminuant la chronaxie nerveuse, ne pouvait donc pas rétablir l'isochronisme, ni par suite la transmission. Par suite, ces résultats négatifs seraient en accord avec la théorie chronologique de la curarisation aussi bien que les résultats positifs. Rushton reconnaît que ses expériences ont bien été faites dans la seconde phase.

Reste, dit-il, entre nos résultats expérimentaux un désaccord qu'il espère voir éclaircir prochainement. Je puis lui donner immédiatement satisfaction.

Rushton pense avoir montré la curarisation (par le curare), sans augmentation de chronaxie musculaire, même avec une électrode correcte (1/4 mm. de diamètre) dans la condition suivante. Un couturier est excisé avec son nerf, et on mesure sa chronaxie; on l'abandonne à lui-même pendant une ou plusieurs heures et on recommence la mesure; on trouve une chronaxie à peu près doublée, pourtant l'excitabilité indirecte subsiste; par conséquent il y a un hétérochronisme sans curarisation; on ajoute un peu de curare; l'excitabilité indirecte disparaît en quelques minutes sans nouvel accroissement notable de la chronaxie musculaire. Or, nous avons constaté le fait suivant que j'ai indiqué, mais très brièvement et incidemment (1934 *a*, p. 119) de sorte qu'il a pu légitimement échapper à Rushton. Un couturier conservé dans les conditions ci-dessus et étant devenu hétérochrone à la limite de la curarisation peut donner encore un petit nombre de réponses à la stimulation de son nerf, avant de cesser de répondre. Rushton dans ses expériences, essayait *une fois* l'excitabilité indirecte, ajoutait le curare et répétait la stimulation

du nerf *une fois par minute*; au bout de 1 à 7 minutes, cette stimulation devenait inefficace. Je traduis: le muscle n'était plus capable de répondre qu'à 1, 2, …, 7 stimulations, après quoi il a cessé de répondre en raison de son altération antérieure, non à cause du curare, qui était en quantité beaucoup trop petite pour avoir aucune action sensible à ce moment; en effet les doses employées par Rushton auraient été incapables de curariser même au bout d'une heure, et le résultat qu'il leur attribue s'obtient en un temps invraisemblablement court pour quelque dose que ce soit.

Je suis persuadé que Rushton aurait obtenu le même résultat s'il avait fait la contre-épreuve de stimuler de la même manière sans ajouter du curare. S'il veut bien la faire (dans la même saison où il a fait ses expériences, car le couturier a des propriétés saisonnières), et s'il veut bien prendre enfin en considération l'argument des muscles lents, je puis espérer que Rushton se trouvera d'accord avec moi.

En attendant, il est intéressant tout de même d'éclaircir certains points soulevés dans son article, et qui paraîtraient subsister comme objections.

1e objection—La démonstration par les courants progressifs ne prouve rien, sinon qu'après curarisation, on a affaire à une excitabilité plus lente qu'avant, ce qui est le fait banal de l'excitabilité musculaire révélée par paralysie de l'excitabilité nerveuse—*Discussion:* une première fois (1933) Rushton avait trouvé étrange l'affirmation de la relation classique entre la chronaxie et l'inefficacité des courants progressifs; il avait même invoqué contre cette relation une expérience de Keith Lucas qui, en réalité, la vérifie, mais que Rushton interprétait à contre-sens. Cette fois, il déclare mon expérience ingénieuse, mais ne la comprend pas, puisqu'elle consiste précisément à montrer que l'excitabilité lente ne préexistait pas à la curarisation.

2e objection—Comment se fait-il que la strychnine appliquée sur le nerf n'arrête pas la transmission, tandis qu'elle l'arrête quand elle est appliquée sur toute la préparation, l'hétérochronisme étant le même dans les deux cas? Sans doute, il a été démontré que dans le premier cas, il y a transition graduelle d'une chronaxie à l'autre, mais dans le second, que se passe-t-il dans la petite terminaison nerveuse non myélinisée contiguë au muscle?—*Discussion:* dans le premier cas la transition s'effectue graduellement sur une longueur de l'ordre du centimètre; dans le second, si gradation il y a (ce que je ne crois pas) elle s'effectue sur une longueur inférieure au centième de millimètre. Tout cycliste ou automobiliste conviendra qu'un escarpement de quelques mètres de haut avec une pente à 45° est un obstacle pratiquement infranchissable tandis que la même différence de niveau sur une distance de quelques kilomètres est insignifiante.

3e objection—La chronaxie nerveuse diminuée par action des centres (chronaxie de subordination) crée un hétérochronisme avec le muscle innervé; "thus…the law of isochronism is valid only for excised preparations, and does not apply to animals in possession of their higher centres"—*Discussion:* J'avoue que j'ai été quelque temps embarrassé par ce cas qui me paraissait en effet un singulier paradoxe. Mais l'analyse pénétrante qu' A. M. Monnier et H. H. Jasper ont faite du mécanisme de la subordination a montré expérimentalement, que le courant d'action du nerf en cet état n'est pas sensiblement plus bref malgré la diminution de chronaxie.

(La théorie physicochimique du phénomène rend d'ailleurs parfaitement compte de cette dissociation entre l'excitabilité et la réaction du nerf.) Monnier et Jasper ont explicité (1932) cette conséquence importante qu'ainsi la subordination change les conditions de réceptivité du neurone, non celles de son action sur l'élément consécutif.

4ᵉ objection. Grundfest, stimulant des fibres musculaires et les fibres nerveuses qui les animent, avec les mêmes électrodes, très petites, a trouvé néanmoins l'ET2R du muscle beaucoup plus grand que celui du nerf—*Discussion :* présenté ainsi, le fait semble nettement en contradiction avec tout ce que j'ai développé sur les petites électrodes; en réalité, Grundfest a obtenu pour le muscle des chronaxies sensiblement normales; c'est l'ET2R du nerf qui était faussé, dans le sens d'une diminution, par suite d'une autre cause d'erreur instrumentale que j'ai expliquée et expérimentalement démontrée (1933 *b* et 1934 *a*). Grundfest n'a, autant que je sache, rien objecté contre ma critique, dont je lui ai envoyé un "reprint" en 1933.

Je crois n'avoir laissé dans l'ombre rien d'important. A mon tour, je pourrais demander à Rushton comment, après avoir rejeté ma théorie, il explique la curarisation. Il insinue, discrètement, que la théorie par l'empoisonnement des terminaisons nerveuses est toujours valable. Je m'étonne qu'un étudiant en physiologie de Cambridge ignore Langley, et sa démonstration que l'action du curare porte *au-delà* de ces terminaisons. Il néglige également la discussion que j'ai faite de mon côté sur l'irréalité de la plaque motrice considérée comme organe distinct, capable de fixer électivement un toxique. Mais passons là-dessus. L'empoisonnement électif de ces organes mythiques ne saurait pas rendre compte des faits qui ressortent de l'analyse de la curarisation; il n'y a pas seulement l'augmentation indéfinie de la chronaxie musculaire, qui a suscité mes premiers doutes et que Rushton niait jusqu'à ces derniers temps; il y a surtout la diversité des hétérochronismes sous l'action des divers agents curarisants, diversité dont Rushton ne dit pas un mot, mais qui est nettement inconciliable avec la vieille théorie classique.

VII. APPLICATION AU FONCTIONNEMENT DES CENTRES NERVEUX.

La théorie classique de la curarisation offrait l'agrément d'une image simple, l'interruption du contact des deux éléments par la suppression fonctionnelle d'une pièce intermédiaire. L'isochronisme conditionnant la transmission de l'activité entre ces deux éléments est plus abstrait; mais n'est-il pas intéressant de ramener ainsi un phénomène vital à une loi générale de la mécanique? A. V. Hill, qui est venu à la Physiologie par la Physique, avait senti l'attrait de cette conception; s'étant laissé (momentanément, j'espère) convaincre par Rushton qu'il fallait l'abandonner, il la regrettait, et en donnait (1933) une appréciation que je demande la permission de reproduire ici.

"The most beautiful application of Lapicque's theory, one which was perhaps a little too convincing because of its beauty, and because it appeared to explain so much, was that of the mechanism by which paralysis is caused by curare and other drugs, or by such agencies as fatigue.... The case was like that of two tuned electric circuits, sending and receiving."

Le rapprochement avec le principe de résonnance, base de la radiophonie, s'impose en effet, mais ce n'est qu'une comparaison assez lâche ; il ne peut y avoir exactement résonnance, puisque l'influx nerveux n'est pas une vibration périodique. Toutefois, il s'agit de relations de même famille, partant du principe évident et déjà vu plus haut que l'efficacité de tout effort dépend de son accord chronologique avec l'effet à produire. Pour ouvrir une porte un peu lourde, lui appliquera-t-on le vif mouvement de main par lequel on lance une petite pierre ?

Le cas particulier de l'influx nerveux peut être précisé. C'est un phénomène *amorti* ; il n'oscille pas autour du potentiel du nerf au repos ; il s'en écarte dans un sens toujours le même, suivant une courbe définie, qui le ramène au niveau de départ sans dépasser celui-ci. Entre perturbations de cette forme, si on n'a pas la véritable résonnance, on a quelque chose d'analogue, que Monnier (1934) vient de définir physiquement et mathématiquement, sous le nom de *pararésonnance* ; deux circuits électriques à ondes amorties s'excitent l'un l'autre avec une efficacité d'autant plus grande que leurs *constantes de temps* (nous pourrions dire leurs chronaxies), sont plus voisines. Autrement dit, pour produire dans le circuit *A* une perturbation d'une grandeur donnée, le circuit *B* devra lui-même être le siège d'une perturbation d'autant plus grande que sa constante de temps sera plus différente. Mais l'influx nerveux est d'une grandeur invariable (loi du "tout ou rien") ; il ne pourra donc pas compenser par un changement de grandeur un hétérochronisme éventuel ; l'isochronisme est alors, non pas seulement la condition optima, mais la condition nécessaire d'efficacité.

L'isochronisme, établi comme fait empirique, passe ainsi à la qualité supérieure de loi rationnelle. Dès lors, il n'est plus limité au cas nerf-muscle ; il vaut pour toute transmission intercellulaire, et notamment pour le passage de l'influx nerveux à travers les centres.

En effet, la révolution apportée à la fin du siècle dernier dans la conception anatomique du système nerveux, par la doctrine du neurone, a substitué la discontinuité à la continuité de l'ancien réseau ; au point de vue fonctionnel, ceci veut dire, comme Sherrington l'a fait remarquer, que les centres se différencient des nerfs en ce que la propagation de l'influx, au lieu d'être intracellulaire, devient intercellulaire. Cette condition particulière doit expliquer la fonction propre des centres ; le lieu de cette fonction n'est plus l'ancienne cellule, aujourd'hui centre trophique organisé pour chaque neurone autour de son noyau (*périkaryone*) ; c'est la surface de jonction du neurone avec son voisin (*synapse*).

Cette analyse aussi juste que pénétrante, formulée de très bonne heure, s'est facilement imposée. Mais elle a subi un développement fâcheux par la façon dont on a voulu attribuer à la synapse le pouvoir de laisser passer ou d'arrêter l'influx. La synapse n'a pas d'existence matérielle distincte ; étant seulement le contact de deux réalités qui sont deux neurones, elle ne peut avoir d'autres propriétés que les résultantes des qualités de ceux-ci.

Voici une comparaison physique ; soit un barreau de cuivre soudé à un barreau d'étain ; la soudure constitue un point singulier doué de propriétés spéciales ; notamment le chauffage de ce point engendre une force électromotrice ; ce phé-

nomène est déterminé par le conflit des qualités des métaux en présence, car si l'on change l'un ou les deux, sa grandeur et même son signe varieront d'une façon qui peut être calculée *à priori* par le rapport d'autres propriétés des métaux. Ici, il est évident d'ailleurs que la soudure n'est pas un être en soi ; nous n'avons que deux substances ; leur contact ne peut pas être matérialisé à part.

Je pense qu'il en est de même pour la synapse ; la conception courante d'une *membrane synaptique* capable, par exemple, de subir des empoisonnements électifs n'est pas seulement une hypothèse gratuite, elle soulève les mêmes difficultés que les *jonctions myoneurales* critiquées par Langley. Quant à charger cette membrane imaginaire, ou, plus vaguement, l'entité "synapse" d'aiguiller l'influx nerveux dans le sens nécessaire à la production d'un mouvement adapté, c'est une explication verbaliste ou, si l'on veut, anthropomorphique, comme celle qui jadis attribuait ce rôle à l'ancienne cellule nerveuse. Et pourtant l'aiguillage, fonction primordiale des centres nerveux, ne peut s'expliquer par des considérations simplement anatomiques. Sherrington l'a clairement montré (1908, p. 85) par l'analyse d'un réflexe classique.

Soit une patte dont on pince un doigt ; la patte se retire ; cela veut dire qu'à tous les segments du membre les muscles fléchisseurs se sont contractés, non les muscles extenseurs. Or Sherrington lui-même l'a expressément constaté, les neurones moteurs des uns et des autres sont situés pêle-mêle sur une certaine longueur de la moelle épinière, et les fibres centripètes venant de la petite région stimulée arrivent elles-mêmes éparses dans la même région. Peut-on, demande Sherrington, admettre que ces fibres sensitives sont allées dans la substance grise de la moelle à la recherche des cellules motrices des fléchisseurs, en laissant de côté les cellules motrices des autres muscles, notamment des extenseurs ?

Assurément non. Nous ne sommes pas capables de distinguer les synapses dans la substance grise, sauf quelques cas particuliers comme le cervelet. Mais certainement, elles ne sont pas aussi simples que nous les présentent généralement les schémas de connexions nerveuses. Les neurones en cause tels qu'ils se révèlent par la méthode de Golgi, tels qu'ils sont reproduits par les auteurs quand il s'agit d'observations et non de théorie, montrent des expansions étalées sur de larges espaces. (Voir Fig. 5.) Évidemment, les contacts sont multiples ; ce que l'on pourrait reconstruire d'après ces formes, ce seraient, non pas les synapses bilatérales des schémas, mais des *polysynapses*. D'ailleurs toutes les fibres sensitives bifurquent en arrivant à la moelle et possèdent des terminaisons étagées. Autrement dit, le système de connexions entre neurones est diffus, au moins dans les centres qui nous occupent. Dans cet enchevêtrement de voies multiples anatomiquement constituées en permanence (l'hypothèse des contacts successivement fermés et rompus par amœboïsme étant abandonnée), il faut pourtant un choix. L'énergie nerveuse, loin de s'affaiblir en diffusant, se régénère en chaque point de son trajet ; si l'influx nerveux, une fois arrivé dans un centre, pouvait se répandre dans tous ces chemins à la fois, il aboutirait à la mise en jeu quasi-simultanée de tous les muscles, des extenseurs comme des fléchisseurs ; on n'aurait pas un mouvement coordonné, mais un spasme de rigidité, comme dans le tétanos ou la crampe strychnique. Mais si

les divers neurones moteurs englobés dans la polysynapse n'ont pas tous la même chronaxie, l'influx centripète accordé avec les uns sera par là même en désaccord avec les autres; il ne pourra transmettre l'excitation qu'aux premiers. Ainsi se dessineront des voies fonctionnellement ouvertes et d'autres fonctionnellement fermées.

J'avais émis cette hypothèse comme principe du mécanisme nerveux (1907) dès que j'eus obtenu les premières justifications théoriques de l'isochronisme; le cas nerf-muscle me laissait encore hésitant, en raison de l'hétérogénéité des deux éléments en cause; mais de neurone à neurone, la loi me paraissait s'imposer *à priori*. Les vérifications ne vinrent que beaucoup plus tard.

Pour les chercher, je m'étais adressé au réflexe de l'animal décapité, croyant ainsi, suivant la tradition de la physiologie expérimentale, simplifier le système nerveux en le réduisant à la moelle. Mais on se trouve alors en présence de la complication suivante: la stimulation doit être réitérée. Les excitabilités de cette
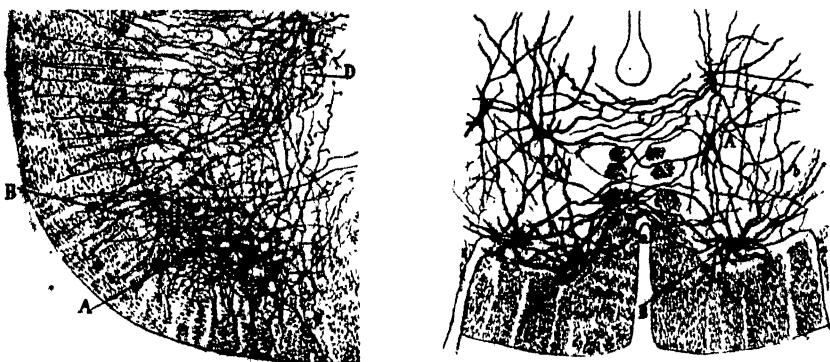

Fig. 5. Deux dessins de Ramon Cajal représentant, dans la moelle du Chat, à gauche, des terminaisons de fibres centripètes; à droite, des perikaryones et leurs dendrites avec lesquels doivent s'articuler ces fibres. (Les lettres se rapportent à des indications de Cajal sans intérêt ici.)

sorte, qui sont fréquentes dans le domaine végétatif (nerfs vaso-moteurs, sécrétoires, etc.) et que j'ai étudiées en détail sous le nom d'*excitabilité itérative*, comportent des relations chronologiques précises, mais beaucoup plus abstraites que l'isochronisme du nerf volontaire avec le muscle strié qui répond au stimulus isolé; il faudrait d'assez longs développements pour expliquer ces relations, qui présentent d'ailleurs un grand intérêt, et commencent à éclairer la façon dont l'encéphale contrôle la moelle.

Nous nous en tiendrons ici au cas du complexe encéphalo-rachidien, où l'on obtient une réponse au stimulus isolé, et qui a fourni une vérification aussi simple qu'inattendue.

Un praticien, Bourguignon (1917, 1923), visant l'électrodiagnostic, s'était mis à la mesure de la chronaxie chez l'Homme: pour reconnaître en chaque cas particulier les écarts de la normale, il avait entrepris, sans idée préconçue, de mesurer les chronaxies de tous les muscles squelettiques chez l'Homme sain. La comparaison de très nombreux chiffres fit apparaître la loi suivante: à chaque segment de

membre, les divers muscles qui concourent à un mouvement ont sensiblement même chronaxie; les muscles antagonistes de ce mouvement présentent aussi une égalité de chronaxie entre eux, mais cette chronaxie est approximativement soit double, soit moitié de la précédente.

Or ces chronaxies dites musculaires, mesurées sur les points moteurs classiques, appartiennent en réalité aux nerfs correspondants particulièrement excitables à l'endroit où ils pénètrent dans le muscle. La constatation de Bourguignon, vérifiée d'ailleurs sur les Vertébrés les plus divers dans des expériences où la stimulation portait directement sur le nerf (L. et M. Lapicque, 1928 a; Rudeanu et Bonvallet, 1932 d), peut donc se formuler schématiquement comme suit: les nerfs des fléchisseurs d'une part, les nerfs des extenseurs d'autre part, diffèrent comme chronaxie environ dans le rapport de 1 à 2. Si, des parties périphériques où elle est mesurable, nous étendons ces chronaxies aux parties centrales des neurones moteurs, extension légitime, puisque la chronaxie est une propriété protoplasmique et non structurale, nous trouvons exactement la condition postulée pour expliquer chronologiquement la discrimination réclamée par Sherrington.

Des voies anatomiquement distinctes feraient du système nerveux quelque chose de comparable à un réseau de télégraphie électrique, avec fils, et c'est sur ce modèle qu'on a classiquement cherché à l'analyser. En réalité, ce système fonctionne sur le principe de la télégraphie sans fil, de la radio, pour laquelle deux appareils semblablement construits étant placés côte à côte et baignés par les mêmes ondes, mais réglés sur deux longueurs d'ondes différentes, l'un pourra être excité pendant que l'autre sera insensible. A la longueur d'onde, on peut, par identité mathématique, substituer la durée de la période, obtenue en divisant par cette longueur les 300,000 kilomètres que parcourt en 1 seconde la perturbation électromagnétique. On voit alors que la condition de fonctionnement d'un poste récepteur, l'accord chronologique (tuning) avec le poste émetteur, est l'égalité de deux durées caractéristiques, à la façon de notre isochronisme.

Je tiens à faire remarquer que la conception de l'isochronisme dans le système nerveux est née de considérations physiques et biologiques se rapportant directement au sujet, nullement d'une métaphore à partir de la radiotélégraphie; il suffit pour s'en convaincre de regarder la date, 1907, où a été formulée cette théorie. La radio, venue ensuite et aujourd'hui familière, fournit simplement une image commode pour évoquer par analogie le mécanisme en cause; elle peut servir encore pour un deuxième aspect de la question.

En tournant le bouton d'un poste récepteur, nous changeons la période sur laquelle il est accordé, et ainsi, par rapport à un poste émissif donné, nous le rendons muet ou nous le faisons chanter.

De même la chronaxie d'un neurone peut être changée par l'action d'un autre neurone. La détermination de certaines voies par accord entre les chronaxies fixes suffirait pour le choix entre extenseurs et fléchisseurs dans le réflexe stéréotypé d'une Grenouille décapitée; elle ne rendrait pas compte de la variabilité qui caractérise les réponses d'un animal à système nerveux entier.

En fait, sur un animal intact, les chronaxies des neurones moteurs médullaires

(mesurées toujours sur leur axone périphérique) dépendent d'une action exercée par un centre ou des centres d'ordre supérieur, dont le principal est situé dans la base de l'encéphale. La différence entre chronaxies d'antagonistes est un effet de cette subordination. Par exemple, chez la Grenouille intacte ou privée seulement de ses hémisphères cérébraux, la chronaxie du nerf du gastrocnémien, muscle extenseur du pied, apparaît généralement deux fois plus petite que celle des nerfs du tibial et du péronier, muscles fléchisseurs; si l'on coupe les nerfs vers leurs racines pour les séparer de la moelle, ou si l'on coupe la moelle au bulbe, pour la séparer de l'encéphale, la chronaxie du premier nerf est doublée (M. Lapicque, 1923). Celle des deux derniers ne changeant guère, on arrive sensiblement à l'égalité. Cette égalisation se produit de même chez les Mammifères et chez les Oiseaux à la suite de diverses lésions des organes encéphaliques, de l'administration d'anesthésiques; dans tous ces cas, la coordination des mouvements disparaît du même coup. A certaines doses, à certaines phases de leur action, les anesthésiques, l'alcool, la morphine inversent le rapport des chronaxies. La subordination peut donc s'exercer dans un sens ou dans l'autre. La démarche titubante de l'ivrogne paraît s'expliquer par les troubles que l'alcool apporte dans la subordination (L. et M. Lapicque, 1928 *a*; Lapicque et Kajiwara, 1930; Rudeanu et Bonvallet, 1932 *a*).

Physiologiquement, la subordination est influencée par voie réflexe. L'innervation proprioceptive du muscle considéré, toutes les innervations centripètes, suivant l'intensité et la durée de leur mise en jeu peuvent renforcer, inverser, supprimer la subordination (Achelis, 1927; L. et M. Lapicque, 1928 *b* et 1934; Rudeanu et Bonvallet, 1932 *d*; L. et M. Lapicque et M. Bonnet, 1935).

La subordination se retrouve chez les Invertébrés, Mollusques (M. Lapicque, 1931) ou Crustacés (H. Frédéricq, 1932; les Chauchard, 1932, 1933, 1934). Cette généralité, malgré les différences profondes dans la constitution de ces divers systèmes nerveux, montre bien qu'il s'agit d'une fonction essentielle.

Chez les Mammifères, le cerveau, particulièrement la zone motrice de l'écorce cérébrale, n'est pas l'origine de la subordination, puisque sa suppression laisse subsister la subordination, comme la coordination des mouvements (Rudeanu et Bonvallet, 1932 *d*). Mais il l'influence quand il commande un mouvement; on vient de le voir clairement en étudiant les chronaxies périphériques au cours du réflexe conditionné. Un chien étant dressé à lever une patte 3 ou 4 secondes après une sonnerie, on a le temps d'intercaler chaque fois un essai de stimulation électrique dans ce temps de latence, et par une série d'essais on obtient des mesures. On observe ainsi des changements réguliers, quantitativement importants, des chronaxies des divers muscles du membre commandé et aussi dans les autres membres, en raison, probablement, d'un réglage d'équilibration du corps (Drabovitch et les Chauchard, 1934).

Toutes ces recherches ont besoin d'être encore poursuivies avant que les diverses modalités de la subordination puissent être systématisées. La question est très complexe, et cela se comprend, puisqu'il s'agit du processus par lequel se constitue l'individualité animale. L'acte nerveux élémentaire, tel que le réflexe de la Grenouille décapitée, n'a point de sens biologique quand on le prend isolément; c'est un

fonctionnement non adapté; pour déclencher immuablement un mouvement de retrait localisé, il serait inutile que l'influx nerveux, né du pincement d'un doigt, remontât jusqu'à la moelle avant de redescendre au muscle voisin de son origine. Mais, sur l'animal à système nerveux intact, la réaction variera suivant les cas, semblera dépendre de l'arbitraire du sujet, manifester une personnalité; en réalité, il y a à chaque instant combinaison de toutes les sensations concomitantes et de toutes les prédispositions acquises ou innées. Ceci est la véritable fonction du système nerveux; cette *action intégrative*, comme Sherrington l'a nommée, ne peut évidemment s'accomplir que par des commandes interneuroniques multiples et fugaces. Les changements de chronaxie par subordination, naguère simple vue de l'esprit pour résoudre abstraitement le problème, constituent aujourd'hui une série déjà longue de constatations expérimentales. Leur déterminisme n'est pas encore entièrement établi; il offre un champ de recherches qui promet d'être fécond.

VIII. SOMMAIRE.

I. Les muscles lents d'Invertébrés comparés aux muscles rapides de la Grenouille et de l'Homme, sous le double point de vue de leur contractilité et de leur excitabilité électrique, ont fait naître l'idée de la chronaxie, c'est-à-dire d'une unité de temps particulière à chaque tissu, réglant simultanément ses divers processus.

II. Une telle unité de temps s'obtient avec une grande précision sur le phénomène artificiel qu'est l'excitation électrique, en considérant pour chaque tissu la façon dont l'intensité liminaire varie en fonction de la durée de passage du courant. Cette relation s'exprime par une courbe qui est remarquablement semblable d'un tissu à un autre, sous réserve d'un ajustement convenable de l'échelle des durées. En prenant conventionnellement pour repère la durée correspondant au double de l'intensité liminaire pour les temps très longs, on obtient la chronaxie *sensu-stricto*.

III. La conception générale de la chronaxie se vérifie chez les végétaux doués de mouvement, notamment entre deux espèces de Sensitives. Mais la relation de la chronaxie au mouvement n'est pas la même de Sensitive à muscle que de muscle à muscle; ce qui se comprend quand on considère la façon différente dont le mouvement dans les deux cas est lié au processus fondamental de l'excitation.

IV. Pour un muscle squelettique de Vertébré, la chronaxie est la même que celle du nerf qui l'anime. Cet isochronisme est une condition nécessaire pour la transmission de l'excitation du nerf au muscle, car chaque fois qu'une perturbation quelconque, intoxication, fatigue, etc., altère soit le muscle, soit le nerf de façon à produire entre eux une différence importante de chronaxie, la transmission est arrêtée. L'action du curare, en particulier, consiste à augmenter la chronaxie musculaire.

V. Les auteurs qui ont cru pouvoir assigner au muscle normal une chronaxie notablement plus grande que celle de son nerf ont eu affaire à de fausses chronaxies, effet des électrodes trop larges qu'ils employaient. Un raisonnement physique assez

simple explique cet effet. En tout cas, le caractère erroné des indications fournies par les électrodes larges apparaît clairement dans ce fait, que ces électrodes n'indiquent aucune différence dans l'excitabilité pour les muscles lents ou rapides.

VI. Diverses objections secondaires contre l'isochronisme et le mécanisme chronaxique de la curarisation sont discutées.

VII. Le mouvement le plus simple, tel que la rétraction d'un membre, suppose un choix entre extenseurs et fléchisseurs; la disposition anatomique des éléments nerveux ne fournit pas d'explication de ce choix. Mais l'expérience montre, quand la moelle a gardé ses connections fonctionnelles avec les centres encéphaliques, une différence de chronaxie importante et systématique, entre les motoneurones des fléchisseurs, d'une part, des extenseurs, d'autre part. L'influx nerveux peut donc être aiguillé par isochronisme dans l'un des systèmes et arrêté dans l'autre par hétérochronisme.

Cette différence de chronaxie résulte d'une action des centres encéphaliques sur les neurones médullaires (subordination); cette action varie en grandeur et même en signe suivant des influences réflexes ou psychomotrices; l'aiguillage chronaxique est donc essentiellement variable et peut expliquer la diversité des réactions animales.

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